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EVAPORATION IN AGRICULTURAL METEOROLOGY[1]

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The rate of evaporation from a free water surface and its relation to other meteorological factors are of considerable importance to agricultural science. The correlation of evaporation with air temperature, vapour pressure, and wind velocity has been examined by Hopkins and James (1), who showed that while, in general, high average temperatures were associated with high evaporation, the correlation between the two quantities was very poor. The object of the present note is to emphasize the fact that in order to relate the rate of evaporation to other meteorological factors, the temperature of the evaporating liquid surface must be known, and that the measurement of such temperatures at agricultural meteorological stations would be valuable.
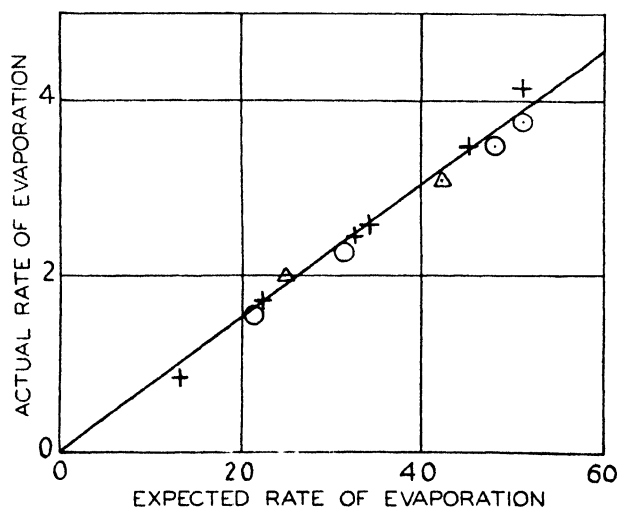

FIG. 1. *Relation between expected* (E_1) *and actual* (E_2) *rates of evaporation of water, in arbitrary units.* +, *air and water at same temperature.* O, *water warmer than air.* △, *air warmer than water.*

On theoretical grounds the rate of evaporation E under any given conditions should be directly proportional to the quantity $(P - p)$, where P is the saturated vapour pressure at the temperature of the surface of the evapor-

ating liquid and p is the pressure of the vapour actually present in the air. In the course of other work, an opportunity arose of examining experimentally the relation between E and $(P - p)$. A stream of dry air was drawn at constant speed over the evaporating surface. In each experiment the temperature of the liquid in the actual evaporating surface was measured by means of a thermocouple immersed in the surface; a second thermocouple enabled the air temperature just above the evaporating liquid to be measured. These temperatures were measured at regular intervals during the experiment. As they varied somewhat during the course of the experiment, $(P - p)$ would change. However, if dE_1 is the amount of evaporation to be expected in a short time dt, then

$$dE_1 = k(P - p)dt$$

and

$$E_1 = k \int_0^T (P - p)dt$$

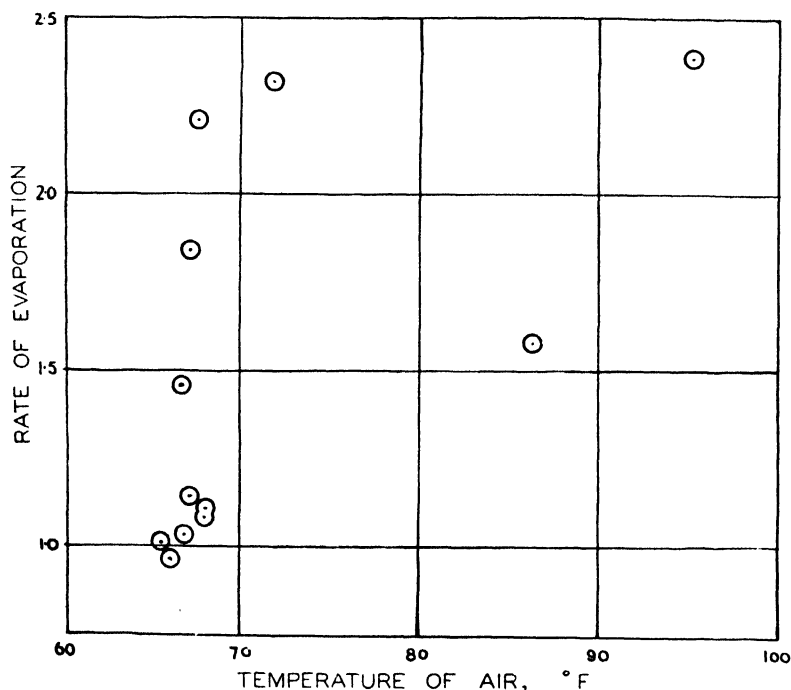where k is a constant and T is the duration of the experiment.

FIG. 2.   *Relation between air temperature and rate of evaporation of water, in arbitrary units.*

This integral was evaluated graphically and the result plotted against the actual rate (E_2) of evaporation as determined directly by weighing. The results are shown in Fig. 1. It will be seen that there is an excellent linear

relation between E_1 and E_2. Since in evaporation experiments in the open air the liquid may be either warmer or cooler than the air above it, experiments were performed with air and water originally at the same temperature ($+$), with the water warmer than the air (O), and with the air warmer than the water ($\triangle$). Fig. 1 indicates that E_1/E_2 remains constant no matter what may be the relative temperatures of air and water.

For comparison, in Fig. 2, the rate of evaporation in these experiments is plotted against the air temperature. Clearly there is no very definite relation between these two quantities. For studies of evaporation it therefore seems very desirable that water surface temperatures should be determined as well as air temperatures. It should be noted that as the evaporation process itself cools the surface, the temperature of the latter is never equal to that of the liquid as a whole. No doubt a good approximation to the required surface temperature could be obtained by floating a thermometer horizontally in the surface.

References

1. HOPKINS, J. W. and JAMES, MABEL F. Can. J. Research, C, 13 : 191-201. 1935.

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AGRICULTURAL METEOROLOGY: SOME CHARACTERISTICS OF WINDS IN ALBERTA AND SASKATCHEWAN[1]

By J. W. Hopkins[2]

Abstract

Over a period of years, the monthly mean mileage of wind per day during the six months, April to September, at four meteorological stations in central and southern Alberta and Saskatchewan is greatest in April and May, and least in July and August. In all cases the range of variation of the monthly means is considerable, being of the order of 50%. Significant correlation in the inter-annual fluctuations at the four stations is not demonstrable from the available data, but there is some indication of correlation between the mileages at a given station in successive months of the same year. During the season as a whole, northwesterly winds predominate, but in this respect also there are pronounced annual variations.

Variation in the amount of wind from day to day within months is also pronounced, and tables are presented showing for each of the six months the relative frequency of occurrence of different daily mileages.

On the whole, more wind is recorded during the daytime than at night. The hourly averages for all six months show a definite diurnal trend, the maximum being in the vicinity of 3 p.m., but the actual hourly sequence on a given day may deviate markedly from this underlying regularity. There is a slight tendency for days of above-average temperature to have a higher wind mileage, but this is a minor factor in comparison with the uncorrelated variation of both quantities.

In view of the important influence that winds may exert upon growing crops, by affecting transpiration and hence the water requirement (3, 4), as well as by mechanical damage, a statistical study has been made of wind observations for the months April to September inclusive recorded at four representative meteorological stations in central and southern Alberta and Saskatchewan. Similar studies of rainfall and temperature statistics have already been reported (1, 2). The stations in question are Lacombe (central Alberta), lat. 52° 28' N., long. 113° 44' W., alt. 2783 ft.; Calgary (southern Alberta), 51° 2' N., 114° 2' W., 3428 ft.; Battleford (central Saskatchewan) 52° 41' N., 108° 20' W., 1592 ft.; and Qu'Appelle (southern Saskatchewan), 50° 31' N., 103° 56' W., 2115 ft.

Wind mileage is recorded at three of these stations by anemometers of the three-cup type; at Lacombe an older type of four-cup instrument is in use. The height above ground at which the instruments are exposed ranges from about 25 ft. at Qu'Appelle to about 80 ft. at Calgary. In all cases the exposure is reported to be unobstructed. It requires to be noted that in April of 1931 the Calgary anemometer was removed to the local airport, since when the mileages reported have been consistently higher than those prevailing at its former location. Comparable data are available for three of these stations for a period of 14 to 16 years, but it has been ascertained that the anemometer at Lacombe was in a defective condition for some time, probably

from 1931 to 1936. Consequently, only the observations made prior to that time at this point have been included in this study.

The data used were in part extracted from the published Monthly Record of the Meteorological Service of Canada (5), and in part transcribed directly from the original record sheets in the Meteorological archives, by kind permission of Mr. J. Patterson, Controller, to whom thanks are due for making this material available.

Monthly Mileage

Table I summarizes the available records of total monthly mileage, giving the average and maximum and minimum values for the period indicated. As three of the months considered comprise 31 days, and three only 30, all comparisons have been made on the basis of monthly mean mileage per day.

TABLE I

WIND MILEAGE, 1922–1937, EXPRESSED AS MONTHLY MEAN MILEAGE PER DAY

Station*	Statistic	April	May	June	July	August	September
Lacombe	Average	198	210	193	167	144	171
	Maximum	230	234	217	201	176	218
	Minimum	146	186	178	140	123	127
Calgary	Average	194	198	185	158	165	174
	Maximum	258	272	251	233	212	222
	Minimum	140	131	139	91	100	125
Battleford	Average	237	247	215	216	187	200
	Maximum	303	307	261	314	223	261
	Minimum	111	206	163	154	135	154
Qu'Appelle	Average	241	241	212	193	195	216
	Maximum	283	287	276	256	248	256
	Minimum	188	170	157	149	176	182

* *Lacombe, 8 yr. data (1923–1930).   Calgary, 15 yr. data for April, June, July and August, 16 yr. for May and September.   Battleford, 15 yr. data for April, 14 yr. for other months.   Qu'Appelle, 16 yr. data for all months.*

There is a similar seasonal trend in the averages for all four stations, the mean daily mileage being greatest in April and May and least in July and August. The average mileage recorded is higher for all six months at the two Saskatchewan stations than at the Alberta ones, but there is no significant difference in this respect between Lacombe and Calgary or between Battleford and Qu'Appelle. In all cases the range of variation of the monthly means over the period studied is considerable. The average range for the six months is, in terms of the unit of mean mileage per day adopted, 63 at Lacombe, 120 at Calgary, 124 at Battleford and 97 at Qu'Appelle, or 35, 67, 57 and 45%, respectively, of the mean. Clearly, therefore, there may be appreciable annual differences in the incidence of wind per month, and it

is of interest to ascertain the degree of correlation (i) between the mileage recorded at the different stations in the same month, and (ii) between the mileage at the same station in successive months.

The first of these points was investigated by the calculation of correlation coefficients. These are, of course, subject to considerable error owing to the small number of years' records available, and a fairly high value is required for statistical significance. Of the 36 coefficients thus calculated for the six possible pairings of the four stations for each month, only one exceeded the 5% point, *i.e.*, rather less than the chance expectation. It cannot therefore be asserted from these data that there is in fact any correlation in the inter-annual fluctuations of monthly wind mileage at the four stations. This is in contrast to the situation prevailing in the case of temperature and precipitation, both of which were previously found to show significant correlation between stations (1, 2).

Correlation between the total mileage recorded at individual stations in successive months of the same year was investigated by the analysis of variance procedure. The variance of the monthly totals over the period of years for which records were available at each station was separated into components due to average differences between months, average differences between years for the six months considered, and residual, as shown in Table II. (In the computations for Calgary, allowance was made for the

TABLE II

ANALYSIS OF VARIANCE OF MONTHLY WIND MILEAGE, APRIL–SEPTEMBER, 1922–1937

Variance	Lacombe†		Calgary		Battleford		Qu'Appelle	
	D.f.	Mean square	D.f.	Mean square	D.f.	Mean square	D.f.	Mean square
Between years	7	767,700*	13	549,100**	13	3,220,500**	15	1,568,100**
Between months	5	4,299,900**	5	3,618,600**	5	6,833,000**	5	6,046,000**
Residual	35	320,900	63	198,500	65	849,300	75	460,000

† *1923–1930.*
* *Exceeds mean square residual, 5% level of significance.*
** *Exceeds mean square residual, 1% level of significance.*

previously noted change in the position of the anemometer in April, 1931.) Average differences between months, due to the foregoing seasonal trend, are reflected in highly significant mean squares, but for all four stations the mean square between years also significantly exceeds the mean square residual, indicating that annual differences in the total mileage for the six months were rather larger than would be expected from the fluctuations in the individual monthly totals, had these been entirely independent in each year.

So far, attention has been confined to the total mileage of wind per month, irrespective of direction. The partition of this total between the eight points of the compass, N, NW, W, SW, etc., is, however, also recorded, and Fig. 1

shows the average values of these in the form of wind-roses. During the season as a whole, north-westerly winds clearly predominate, but in spring there is also a large mileage from the south-east. This, however, decreases as the season advances, tending to be supplanted by northerly and westerly winds at Calgary and Battleford and by southerly and westerly ones at Lacombe and Qu'Appelle.
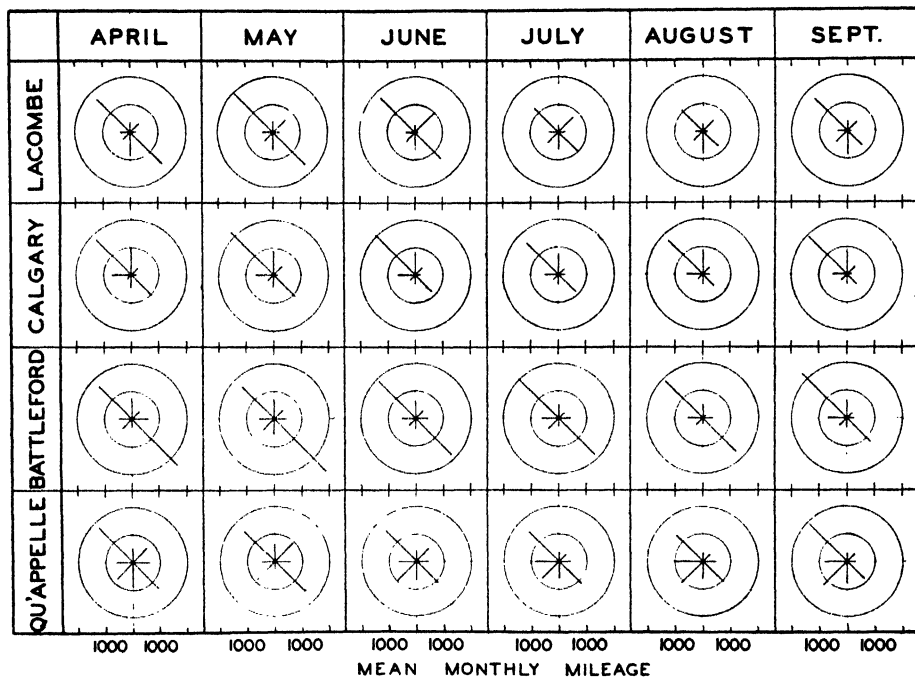

	APRIL	MAY	JUNE	JULY	AUGUST	SEPT.
LACOMBE						
CALGARY						
BATTLEFORD						
QU'APPELLE						

1000 1000   1000 1000   1000 1000   1000 1000   1000 1000   1000 1000
MEAN   MONTHLY   MILEAGE

FIG. 1.   *Mean monthly mileage of wind from the eight points of the compass at meteorological stations in central and southern Alberta and Saskatchewan.*

These data also permit the resultant horizontal air movement at each station each month to be computed. By the application of the ordinary rules for the composition of vectors, the mileages from the eight directions given may be expressed in terms of two components, northerly and westerly. Table III summarizes the values obtained for these, expressed as in Table I in terms of monthly mean mileage per day. There is a preponderance of positive westerly components, *i.e.*, of westerly resultant winds, at all four points, which tends to increase as the season advances, and Calgary is distinguished from the other three stations by the predominance of positive north components. Otherwise, the averages show no clear-cut tendency.

By reference to Table I it will be seen that the averages for both north and west components are small in comparison with the average total mileage. Whilst this is, of course, partly due to the occurrence of winds from opposite directions within the same month, another factor is the annual variation in

TABLE III
RESULTANT WINDS, 1922–1937, EXPRESSED AS MONTHLY MEAN MILEAGE PER DAY

Station	Statistic	April		May		June		July		August		September	
		N	W	N	W	N	W	N	W	N	W	N	W
Lacombe†	Average	− 12	6	4	8	17	− 7	− 5	3	− 7	12	2	33
	Maximum	36	54	50	43	60	37	38	32	14	39	49	46
	Minimum	− 58	− 29	−57	−58	− 16	−39	−40	−18	−47	−10	−29	1
Calgary	Average	38	30	43	38	50	38	28	25	45	34	55	44
	Maximum	85	73	76	85	85	99	77	49	24	74	90	81
	Minimum	− 21	− 5	−14	−14	− 16	10	−18	−10	70	− 4	− 5	18
Battleford	Average	− 9	− 20	−21	−18	13	3	6	16	10	14	30	39
	Maximum	74	65	30	30	54	51	54	77	48	76	59	65
	Minimum	−107	−137	−91	−72	− 30	−69	−39	−34	−22	−26	−41	20
Qu'Appelle	Average	− 6	16	−11	2	−4	27	−12	33	−22	41	−21	41
	Maximum	88	111	116	54	71	92	32	112	29	79	30	81
	Minimum	− 90	− 85	−72	−80	−100	−59	−48	−37	−72	13	−74	14

† *1923–1930.*

the values of both components for all months, which comprises many actual
reversals. The effect of these annual differences on the direction and ampli-
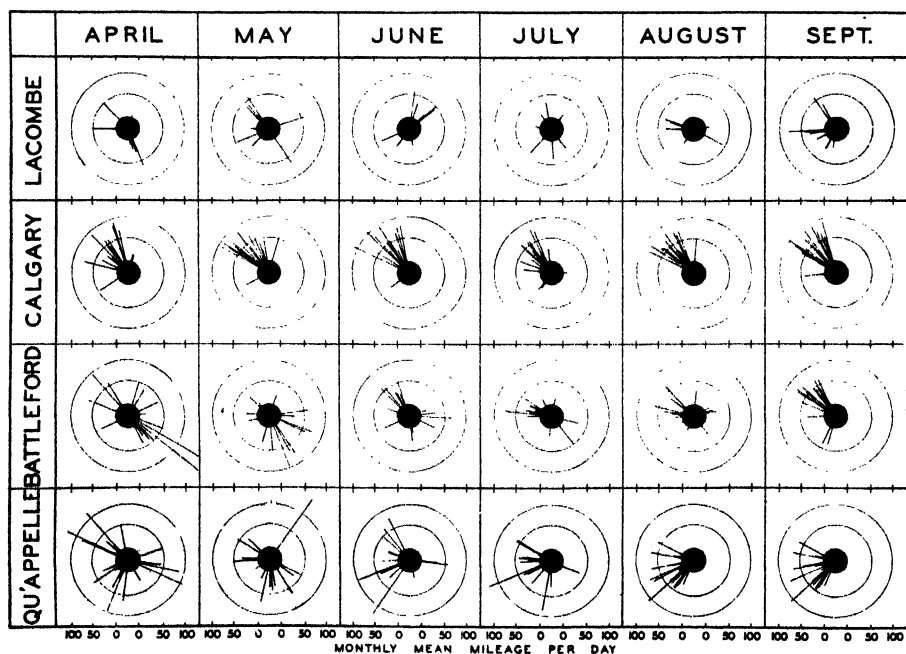tude of the resultant winds is shown in more detail in Fig. 2, in which the

FIG. 2. *Resultant wind vectors. Length of vectors indicates mean mileage per day.*

TABLE IV

ANALYSIS OF VARIANCE OF DAILY WIND MILEAGE, 1922–1937

Station	Variance	April		May		June		July		August		September	
		D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Lacombe†	Inter-annual	7	18294**	7	8040	7	5940	7	17623**	7	10687**	7	20681**
	Intra-annual	219	5627	240	6652	232	6536	236	3560	240	2361	232	4647
Calgary	Inter-annual	12	5221	13	8893	13	4950	12	12769**	13	3541	13	3837
	Intra-annual	405	6937	450	5898	428	4326	419	3300	432	2955	435	4754
Battleford	Inter-annual	13	59813**	13	32279**	13	39349**	13	40063**	13	21607**	13	26510**
	Intra-annual	406	12416	420	14292	405	10930	420	8625	414	6967	396	8650
Qu'Appelle	Inter-annual	13	20313**	13	26331**	13	29875**	13	18146**	13	17577**	13	13294*
	Intra-annual	402	8636	420	7860	406	6482	417	4958	419	5030	405	6037

† 1923–1930.
* Exceeds intra-annual mean square, 5% level of significance.
** Exceeds intra-annual mean square, 1% level of significance.

resultant vector for each month, deduced from the westerly and northerly components, is represented individually. The annual fluctuations in question occur to some extent in all months, but are most pronounced in the first half of the season, April to June.

Daily Mileage

Two aspects of the daily mileage are of particular interest, *viz.*, the variance and frequency distribution characteristic of each month.

Table IV shows the results of an analysis of the total variance of the daily observations for April, May, – – – – September, at each station into components within and between years. On the whole, the mean squares between years tend to exceed those within years, suggesting that the average wind velocity of *e.g.*, July in an individual year is to some extent affected by sources of variation other than those resulting in the day-to-day differences within months. Whilst this is brought out most clearly in the records for Battleford and Qu'Appelle, which are more extensive than those available for Lacombe and more homogeneous than those for Calgary, significant inter-annual variation was demonstrable in the previously examined records of both precipitation and temperature at the Alberta as well as at the Saskatchewan stations (1, 2).

TABLE V

INTRA-MONTHLY STANDARD DEVIATION OF DAILY WIND MILEAGE, 1922–1937

Station	Standard deviation	April	May	June	July	August	Sept.
Lacombe†	Miles per day	75	82	81	60	49	68
	As % of mean	38	39	42	36	34	40
Calgary	Miles per day	83	77	66	57	54	69
	As % of mean	43	39	36	36	33	40
Battleford	Miles per day	111	120	104	93	83	93
	As % of mean	47	48	48	46	45	47
Qu'Appelle	Miles per day	93	89	80	70	71	78
	As % of mean	38	37	37	36	36	36

† *1923–1930.*

Table V shows the intra-monthly standard deviations of daily wind mileage, computed from the intra-annual mean squares of Table IV and indicative of the day-to-day variation experienced within each of the six months. These are given in terms of actual mileage per day and also as a percentage of the mean, often termed the "coefficient of variation". At all four stations the variation in actual mileage per day is greatest in spring (April and May), diminishes progressively throughout the summer, and begins to increase again in September. The mean mileage recorded shows a similar seasonal trend (Table I), with the result that the percentage intra-monthly variation at

Battleford and Qu'Appelle is practically the same for all six months considered. At Lacombe and Calgary, on the other hand, the summer decrease in day-to-day variability is rather more than proportional to the diminution in mean mileage. In all cases the general level of variation indicated is quite high, the standard deviations, comprising roughly one-quarter of the main range, being themselves of the order of 40%, and approaching 50% at Battleford.

Tables VI to IX summarize by months the frequency distribution of the daily mileages recorded at the four stations. The first column of each of these tables indicates the class intervals into which the observations have been grouped, and the second column the average m.p.h. corresponding to the preceding daily totals. This latter figure is useful as a mental reference point, but must, however, be regarded as to some extent conventional, as the average diurnal distribution of wind between the night- and day-time hours is unequal. The main body of each table is then divided into two portions, the first showing for each of the six months separately the percentage frequency of daily totals of the various mileage classes, and the second the percentage (to the nearest unit) of days on which successive values were exceeded.

In all cases the frequency of occurrence of the different daily totals follows a well-defined pattern. Daily mileages of the lowest order, 0 to 60 m.p.d., are rare, but thereafter the number of occurrences in the successive classes rises rapidly to a maximum, from which it diminishes somewhat more gradually as the higher values are traversed. All the frequency distributions thus generated are asymmetrical, the great majority of the observations falling in the lower half of the range of variation and the higher mileages occurring relatively infrequently. This "tail" of high values is much more pronounced in spring than in summer. Thus at Battleford, for example, (Table VIII) 29% of the daily totals recorded for May exceed 300 miles (an average of 12.5 m.p.h.), whereas the corresponding figure for August is only 12%. In this connection, it may be noted that an increase in the incidence of light winds is particularly desirable when crops must be produced under conditions of limited moisture supply, as experiments (3) indicate that the transpiration of plants tends to increase as the square root of the wind velocity, rather than in direct propor- tion to the velocity itself. A reduction in velocity from 7.5 to 5 m.p.h. would therefore cut down the rate of transpiration more than a reduction of from 10 to 7.5 m.p.h.

Not only the general pattern of the frequency distribution, but also its seasonal trend, is similar at all four stations. However, some of the dif- ferences in detail shown in Tables VI to IX may be of considerable practical importance. At Lacombe, central Alberta, for example, only 10% of the August days for which records are available exceed 10 m.p.h. in average wind velocity (Table VI), whereas this value is exceeded in 28% of cases at Qu'Appelle, southern Saskatchewan (Table IX).

Diurnal Variation

Diurnal variation in the incidence of winds was studied by an examination of hourly mileage records. Owing to the labour involved in dealing with the

TABLE VI

FREQUENCY DISTRIBUTION OF DAILY WIND MILEAGE, LACOMBE, 1923–1930

Daily mileage	Average m.p.h.	Percentage frequency of occurrence						Percentage frequency with which exceeded					
		April	May	June	July	August	Sept.	April	May	June	July	August	Sept.
0– 60	0– 2.5	0.4	0.0	0.0	0.0	0.8	1.3	>99	100	100	100	>99	99
60–120	2.5– 5.0	11.0	7.9	12.5	25.4	36.3	26.5	89	92	88	75	63	72
120–180	5.0– 7.5	34.6	32.1	40.6	38.9	41.3	34.8	54	60	47	36	22	37
180–240	7.5–10.0	29.3	30.8	24.4	23.8	16.7	21.7	25	29	22	12	5	16
240–300	10.0–12.5	13.7	17.5	14.4	9.0	3.2	10.4	11	12	8	3	2	5
300–360	12.5–15.0	8.4	7.3	4.2	1.6	1.6	2.9	3	4	4	1	—	2
360–420	15.0–17.5	0.9	1.6	1.9	0.8	0.0	2.1	2	3	2	<1	—	<1
420–480	17.5–20.0	1.3	1.2	0.8	0.4	0.0	0.4	<1	2	1	—	—	—
480–540	20.0–22.5	0.4	0.8	0.4	0.0	0.0	0.0	—	<1	<1	—	—	—
540–600	22.5–25.0	0.0	0.8	0.4	0.0	0.0	0.0	—	—	<1	—	—	—
600–660	25.0–27.5	0.0	0.0	0.4	0.0	0.0	0.0	—	—	—	—	—	—

TABLE VII

FREQUENCY DISTRIBUTION OF DAILY WIND MILEAGE, CALGARY, 1923–1937

Daily mileage	Average m.p.h.	Percentage frequency of occurrence						Percentage frequency with which exceeded					
		April	May	June	July	August	Sept.	April	May	June	July	August	Sept.
0– 60	0– 2.5	1.4	0.4	0.4	2.8	0.7	1.2	99	>99	>99	97	>99	99
60–120	2.5– 5.0	14.4	14.5	14.2	25.4	27.9	25.6	84	85	85	72	71	73
120–180	5.0– 7.5	31.3	33.5	38.5	37.4	38.1	35.6	53	52	47	34	33	38
180–240	7.5–10.0	28.5	25.7	25.2	21.6	19.2	23.1	24	26	22	13	14	14
240–300	10.0–12.5	13.6	14.8	13.8	8.4	8.9	8.0	11	11	8	4	5	6
300–360	12.5–15.0	5.7	6.5	4.1	3.5	4.3	4.1	5	5	4	1	1	2
360–420	15.0–17.5	2.6	2.2	3.4	0.5	0.7	1.8	2	2	<1	<1	<1	<1
420–480	17.5–20.0	1.0	0.9	0.0	0.0	0.2	0.2	2	2	<1	<1	—	<1
480–540	20.0–22.5	0.5	0.9	0.0	0.0	0.0	0.0	1	1	<1	<1	—	<1
540–600	22.5–25.0	0.2	0.2	0.4	0.5	0.0	0.2	<1	<1	—	—	—	<1
600–660	25.0–27.5	0.2	0.0	0.0	0.0	0.0	0.0	<1	<1	—	—	—	<1
660–720	27.5–30.0	0.2	0.4	0.0	0.0	0.0	0.2	<1	—	—	—	—	—
720–780	30.0–32.5	0.0	0.0	0.0	0.0	0.0	0.0	<1	—	—	—	—	—
780–	32.5–	0.2	0.0	0.0	0.0	0.0	0.0	—	—	—	—	—	—

TABLE VIII

FREQUENCY DISTRIBUTION OF DAILY WIND MILEAGE, BATTLEFORD, 1924-1937

Daily mileage	Average m.p.h.	Percentage frequency of occurrence						Percentage frequency with which exceeded					
		April	May	June	July	August	Sept.	April	May	June	July	August	Sept.
0– 60	0– 2.5	2.9	1.2	1.9	1.6	1.9	1.0	97	99	98	98	98	99
60–120	2.5– 5.0	12.9	10.3	18.4	16.9	23.5	17.0	84	88	80	81	75	82
120–180	5.0– 7.5	19.3	21.8	23.9	30.3	30.1	34.8	65	67	56	51	44	47
180–240	7.5–10.0	21.5	21.1	21.5	20.5	21.6	20.5	43	46	34	31	23	27
240–300	10.0–12.5	17.6	16.9	13.5	13.9	10.5	12.2	26	29	21	17	12	14
300–360	12.5–15.0	12.5	10.5	11.3	9.3	8.1	7.7	13	18	10	7	4	7
360–420	15.0–17.5	5.8	8.9	4.1	4.6	3.6	3.9	7	9	5	3	1	3
420–480	17.5–20.0	3.5	4.1	3.1	1.4	0.5	1.3	4	5	2	1	<1	2
480–540	20.0–22.5	1.9	2.5	0.7	0.5	0.2	1.0	2	3	2	<1	<1	1
540–600	22.5–25.0	1.0	1.6	1.1	0.7	0.0	0.2	1	1	<1	<1	—	<1
600–660	25.0–27.5	1.0	0.9	0.6	0.2	0.0	0.5	<1	<1	<1	<1	—	<1
660–	27.5–	0.2	0.2	0.0	0.0	0.0	0.0	—	—	—	—	—	—

TABLE IX

FREQUENCY DISTRIBUTION OF DAILY WIND MILEAGE, QU'APPELLE, 1922–1937

Daily mileage	Average m.p.h.	Percentage frequency of occurrence						Percentage frequency with which exceeded					
		April	May	June	July	August	Sept.	April	May	June	July	August	Sept.
0– 60	0– 2.5	0.2	0.9	0.5	0.0	0.2	0.2	>99	>99	>99	100	>99	>99
60–120	2.5– 5.0	7.3	6.0	9.5	12.5	12.5	10.3	92	93	90	88	87	90
120–180	5.0– 7.5	20.3	23.4	27.4	38.6	34.2	25.3	72	70	63	49	53	64
180–240	7.5–10.0	25.0	22.0	31.9	26.3	24.7	30.7	47	48	31	26	28	34
240–300	10.0–12.5	21.2	23.8	17.3	13.5	21.0	20.8	26	24	13	9	7	13
300–360	12.5–15.0	13.3	12.9	0.9	6.8	5.0	7.5	13	11	6	2	2	5
360–420	15.0–17.5	7.7	6.6	3.7	0.8	1.0	3.6	5	4	3	1	1	2
420–480	17.5–20.0	3.7	3.5	1.9	1.2	1.2	1.2	1	1	1	<1	<1	<1
480–540	20.0–22.5	0.7	0.7	0.5	0.0	0.0	0.2	<1	<1	<1	<1	<1	<1
540–600	22.5–25.0	0.2	0.0	0.5	0.2	0.2	0.2	<1	<1	<1	—	—	—
600–660	25.0–27.5	0.2	0.2	0.0	0.0	0.0	0.0	—	—	—	—	—	—

number of individual observations concerned, two stations only were selected for this purpose, *viz.*, Lacombe (central Alberta) and Qu'Appelle (southern Saskatchewan).

Table X shows for each month separately the average for the four years 1924, 1928, 1932 and 1936 of the mileage recorded during successive hours of the day at Qu'Appelle. The hourly averages for all six months show a definite diurnal trend, increasing during the forenoon and early afternoon until a maximum is attained in the vicinity of 3 p.m., then falling off markedly until about 10 p.m., after which there is little substantial change until some time subsequent to sunrise the next morning. In these respects the average

TABLE X

DIURNAL TREND OF AVERAGE WIND MILEAGE AT QU'APPELLE, SASKATCHEWAN, COMPUTED
FROM HOURLY RECORDS FOR 1924, 1928, 1932, AND 1936

Hourly interval	Average mileage recorded					
	April	May	June	July	August	September
12–1 a.m.	8.1	7.1	6.1	6.5	6.8	7.5
1–2	8.0	7.0	6.3	6.7	6.8	7.4
2–3	8.1	7.1	6.5	6.5	6.7	7.5
3–4	8.1	7.2	6.3	6.4	6.9	7.6
4–5	8.0	7.4	6.5	6.5	7.0	7.8
5–6	7.9	7.3	6.6	6.5	6.9	7.9
6–7	8.6	7.7	7.2 ·	6.6	7.1	7.9
7–8	9.1	8.3	7.6	7.0	7.2	8.1
8–9	9.9	9.7	8.3	7.8	8.0	8.7
9–10	10.5	10.6	9.0	8.5	8.7	9.3
10–11	11.2	11.5	9.7	8.9	9.3	9.9
11–12	11.3	11.8	9.6	9.3	9.9	10.2
12–1 p.m.	11.3	11.9	9.9	9.6	10.3	10.7
1–2	11.8	11.9	10.1	9.8	10.5	10.9
2–3	11.7	12.1	10.1	9.9	10.5	11.2
3–4	11.4	12.3	9.7	9.7	10.3	11.2
4–5	10.7	11.7	9.4	9.6	9.4	10.7
5–6	10.0	11.3	9.0	9.1	8.7	9.3
6–7	9.2	10.4	8.0	8.4	7.6	8.1
7–8	8.6	9.1	7.4	7.4	6.9	7.5
8–9	7.8	7.8	6.6	6.8	6.1	6.9
9–10	7.5	6.9	6.1	6.4	6.0	7.0
10–11	7.6	6.8	6.0	6.3	6.3	7.1
11–12	7.6	6.7	6.1	6.9	6.5	7.4

TABLE XI

PROPORTION OF AVERAGE WIND MILEAGE AT QU'APPELLE, SASKATCHEWAN, FOR 1924, 1928,
1932 AND 1936, RECORDED IN DIFFERENT QUARTERS OF THE DAY

Interval	April	May	June	July	August	September
12–6 a.m.	21.6%	19.4%	20.4%	20.9%	21.6%	22.0%
6–12 a.m.	27.0	26.9	27.3	25.7	26.4	26.0
12–6 p.m.	29.8	32.1	30.9	30.8	31.3	30.8
6–12 p.m.	21.6	21.6	21.4	22.6	20.7	21.2

diurnal march of wind mileage is very similar to that of air temperature (2), but whereas the amplitude of the diurnal variation in air temperature is greater in summer than in spring, the reverse situation obtains in the case of wind mileage.

It follows from the diurnal trend indicated that on the average a greater mileage of wind is recorded during the daytime than at night. The extent of this inequality is illustrated in Table XI, which shows the proportion of the average daily total mileage recorded in different quarters of the day. This is a feature of some practical significance, as shown by the experiments of Martin and Clements (3), which indicated that the effect of wind in accelerating the transpiration of sunflowers was about 20 times greater during the day, when the leaf stomata were open, than at night, when they were closed. It may also be noted that the third quarter of the day, having the highest average wind mileage, is also on the average the period of maximum temperature and minimum relative humidity.

Although, as was mentioned previously, the actual amplitude of the diurnal variation in average wind mileage is somewhat greater in spring than in summer, the percentage variation is much the same for all six months.

Tables XII and XIII, which are similar in form to Tables X and XI, show the averages obtained for Lacombe. These are computed from only two years'

TABLE XII

DIURNAL TREND OF AVERAGE WIND MILEAGE AT LACOMBE, ALBERTA, COMPUTED FROM HOURLY
RECORDS FOR 1924 AND 1928

Hourly interval	Average mileage recorded					
	April	May	June	July	August	September
12–1 a.m.	5.4	6.0	5.1	5.4	4.4	5.4
1–2	5.3	6.5	5.5	5.4	4.5	5.5
2–3	5.0	5.6	5.0	5.3	4.5	5.5
3–4	5.2	5.4	5.0	5.3	4.4	5.8
4–5	5.6	6.2	5.1	5.7	4.9	6.1
5–6	5.9	6.4	5.4	6.1	4.6	5.7
6–7	6.1	7.4	6.6	6.2	4.3	5.5
7–8	6.9	9.3	7.5	7.4	5.5	6.4
8–9	7.5	9.2	8.0	8.0	5.6	7.6
9–10	8.8	10.7	9.0	8.4	6.5	8.4
10–11	10.1	11.3	9.6	8.7	7.9	9.6
11–12	9.6	11.4	10.0	9.2	7.9	10.0
12–1 p.m.	9.9	11.6	10.3	9.2	8.0	9.6
1–2	9.7	11.5	10.0	9.5	8.2	9.6
2–3	9.0	10.4	9.5	8.9	8.4	9.5
3–4	9.2	11.3	9.6	8.7	8.1	10.0
4–5	9.1	11.4	9.4	8.8	8.1	9.4
5–6	8.6	11.0	9.6	8.7	6.8	7.7
6–7	7.4	9.3	8.5	7.7	5.6	6.2
7–8	6.7	7.4	6.8	6.9	5.2	5.8
8–9	6.0	6.0	6.1	6.4	4.4	5.4
9–10	6.0	6.4	5.6	6.4	4.5	5.8
10–11	6.4	6.6	5.8	5.7	4.5	5.7
11–12	5.7	6.1	5.7	5.9	4.4	5.4

records, 1924 and 1928, owing to the subsequently defective condition of the anemometer. For this reason, the hourly means are somewhat more irregular than those for Qu'Appelle, but the general trend is substantially the same.

TABLE XIII

PROPORTION OF AVERAGE WIND MILEAGE AT LACOMBE, ALBERTA, FOR 1924 AND 1928, RECORDED
IN DIFFERENT QUARTERS OF THE DAY

Interval	April	May	June	July	August	September
12–6 a.m.	18.5%	17.7%	17.4%	19.1%	19.3%	19.8%
6–12	28.0	29.0	28.4	27.5	26.7	27.7
12–6 p.m.	31.7	32.9	32.7	30.9	33.7	32.5
6–12	21.8	20.4	21.5	22.5	20.3	20.0
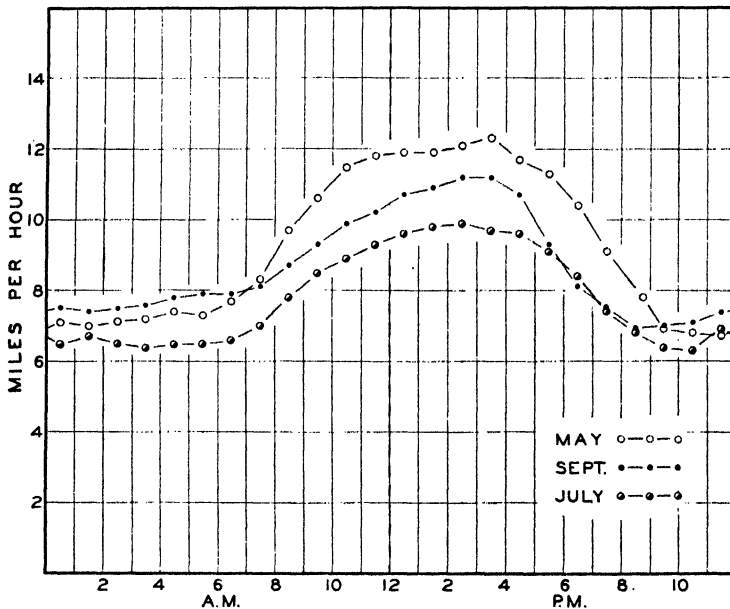
FIG. 3. *Average diurnal wind mileage curves for May, July and September at Qu'Appelle, Saskatchewan, computed from hourly records for 1294, 1928, 1932 and 1936.*

The foregoing remarks apply, of course, to the average values of Tables X to XIII, which are in each case the mean of 120 to 124 daily observations at Qu'Appelle or 60 to 62 at Lacombe. It would, however, be erroneous to suppose that the average trends indicated are closely or even approximately reproduced on the majority of individual days. In practice, the incidence of winds at any specified point is considerably affected by the passage of the successive high and low pressure systems which are such a characteristic feature of the climate of temperate regions, as well as by more local convectional effects, and these may operate to modify or to mask completely

the underlying trends noted above. This will be appreciated from a comparison of Fig. 3, which illustrates the diurnal course of the averages of Table **X** for the three months, May, July and September, with Fig. 4, which shows the actual sequence of hourly values recorded during the first 28 days of July, 1932. The contrast between Figs. 3 and 4 incidentally provides a good example of the somewhat abstract character of many so-called meteorological "normals".
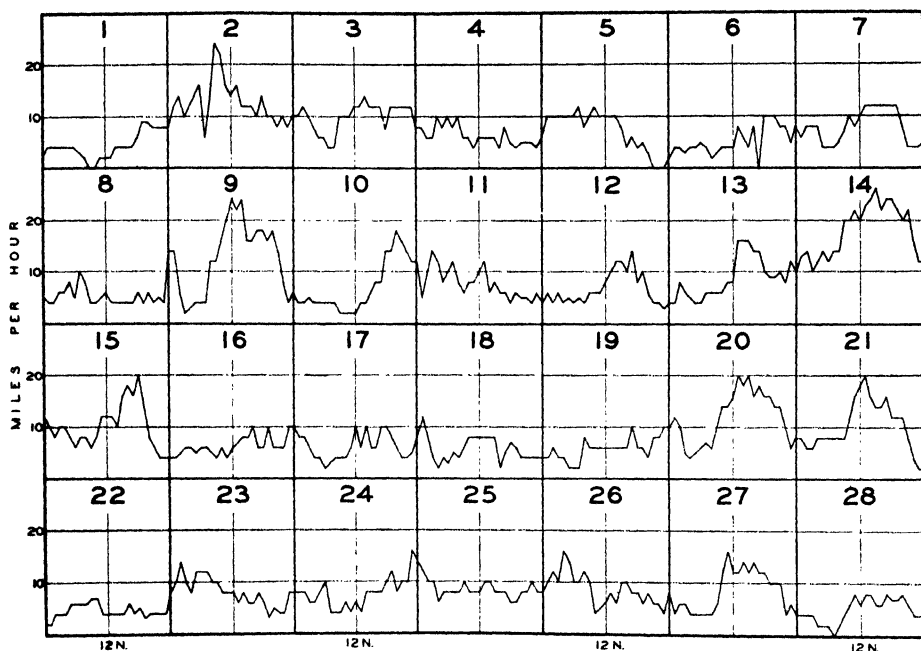
FIG. 4. *Hourly mileage of wind recorded at Qu'Appelle, Saskatchewan, during the first 28 days of July, 1932.*

Hourly Mileage

The individual values used in the computation of the foregoing diurnal trends also provide material for the study of the hourly frequency distributions characteristic of each month. Tables XIV and XV accordingly show the average percentage frequency of occurrence of different hourly mileages during the four and two representative years at Qu'Appelle and Lacombe respectively.

As in the case of the daily totals classified in Tables VI–IX, the frequency distribution of the hourly mileages follows a well-defined pattern. Very low values are again infrequent, and the majority of the observations once more fall in the lower half of the range of variation, being exceeded by a "tail" of higher mileages which diminishes progressively from April–May to August. The resulting skewness of the distributions is, however, less marked than in the case of the daily totals, being accentuated in the latter by the tendency

TABLE XIV

FREQUENCY DISTRIBUTION OF HOURLY WIND MILEAGE, QU'APPELLE, 1924, 1928, 1932 AND 1936

Hourly mileage	Percentage frequency of occurrence						Percentage frequency with which exceeded					
	April	May	June	July	August	Sept.	April	May	June	July	August	Sept.
0– 1.5	2.8	3.2	3.6	1.7	1.2	0.9	97	97	96	98	99	99
1.5– 3.5	8.1	8.0	10.2	9.4	9.8	6.5	89	89	86	89	89	93
3.5– 5.5	15.4	14.5	20.5	20.7	22.6	18.3	74	74	66	68	66	74
5.5– 7.5	15.5	15.9	18.2	20.4	20.6	20.4	58	58	48	48	46	54
7.5– 9.5	13.0	14.3	15.5	16.0	15.2	17.4	45	44	32	32	31	36
9.5–11.5	12.0	12.5	12.1	12.1	11.5	12.1	33	32	20	20	19	25
11.5–13.5	11.0	10.3	8.2	9.3	6.2	9.0	22	21	12	10	13	16
13.5–15.5	7.6	7.8	4.8	5.2	5.0	6.4	15	14	7	5	8	9
15.5–17.5	5.8	5.0	2.9	2.7	3.5	3.8	9	8	4	2	4	6
17.5–19.5	3.7	3.6	1.4	1.1	2.0	2.1	5	5	3	1	2	4
19.5–21.5	2.8	2.2	1.5	0.5	1.4	1.4	2	3	1	<1	1	2
21.5–23.5	1.2	1.4	0.7	0.4	0.3	0.7	1	1	<1	<1	<1	1
23.5–25.5	0.7	0.6	0.3	0.2	0.2	0.4	<1	<1	<1	<1	<1	1
25.5–27.5	0.2	0.4	0.0	0.1	0.3	0.3	<1	<1	<1	<1	<1	<1
27.5–29.5	0.2	0.2	0.1	0.1	0.2	0.2	—	—	—	—	<1	—
29.5–31.5	0.0	0.1	0.1	0.0	0.1	0.0	—	—	—	—	<1	—
31.5–33.5	0.0	0.0	0.0	0.0	0.1	0.0	—	—	—	—	—	—

TABLE XV

FREQUENCY DISTRIBUTION OF HOURLY WIND MILEAGE, LACOMBE, 1924 AND 1928

Hourly mileage	Percentage frequency of occurrence						Percentage frequency with which exceeded					
	April	May	June	July	August	Sept.	April	May	June	July	August	Sept.
0– 1.5	6.4	4.6	4.8	4.7	8.2	5.6	94	95	95	95	92	94
1.5– 3.5	14.8	12.7	14.7	14.2	21.6	16.2	79	83	80	81	70	78
3.5– 5.5	18.6	18.2	20.6	24.2	28.0	21.7	60	64	60	57	42	56
5.5– 7.5	17.8	16.7	17.5	21.4	15.8	22.2	42	48	42	36	26	34
7.5– 9.5	14.6	12.7	13.2	11.4	11.6	10.8	28	35	29	24	15	24
9.5–11.5	11.4	9.8	11.1	8.6	5.6	7.0	16	25	18	16	9	16
11.5–13.5	6.2	7.5	6.9	5.6	3.7	4.2	10	18	11	10	6	12
13.5–15.5	4.0	6.8	5.7	3.4	1.8	4.0	6	11	6	6	4	8
15.5–17.5	2.6	3.6	2.8	2.0	1.5	3.4	4	7	3	4	2	5
17.5–19.5	1.4	2.4	1.2	1.0	0.6	2.2	2	5	2	4	2	3
19.5–21.5	1.0	1.7	0.8	1.2	0.4	1.4	1	3	<1	2	1	1
21.5–23.5	0.6	0.4	0.2	0.8	0.6	0.6	<1	3	<1	2	<1	<1
23.5–25.5	0.2	0.8	0.2	0.7	0.2	0.4	<1	2	<1	1	<1	<1
25.5–27.5	0.1	0.7	<0.1	0.4	0.2	0.0	<1	1	<1	<1	<1	<1
27.5–29.5	0.1	0.2	<0.1	0.2	0.2	0.1	—	1	—	<1	<1	<1
29.5–31.5	0.0	0.4	0.0	0.1	0.0	0.0	—	<1	—	<1	<1	<1
31.5–33.5	0.0	0.3	0.0	0.1	0.0	0.1	—	<1	—	<1	<1	—
33.5–35.5	0.0	0.2	0.0	0.0	0.0	0.0	—	—	—	—	<1	—
35.5–37.5	0.0	0.0	0.0	0.0	<0.1	0.0	—	—	—	—	—	—

of high velocities to persist over a number of successive hours of the same day (cf. Fig. 4). This tendency is most noticeable during the summer months, and hence affects the seasonal trend also, so that this is less pronounced in the hourly than in the daily mileages, although still quite noticeable.

Figs. 5 and 6 show by means of histograms both the average frequency distributions of Tables XIV and XV and also those for each year separately, the four- and two-year averages for Qu'Appelle and Lacombe respectively
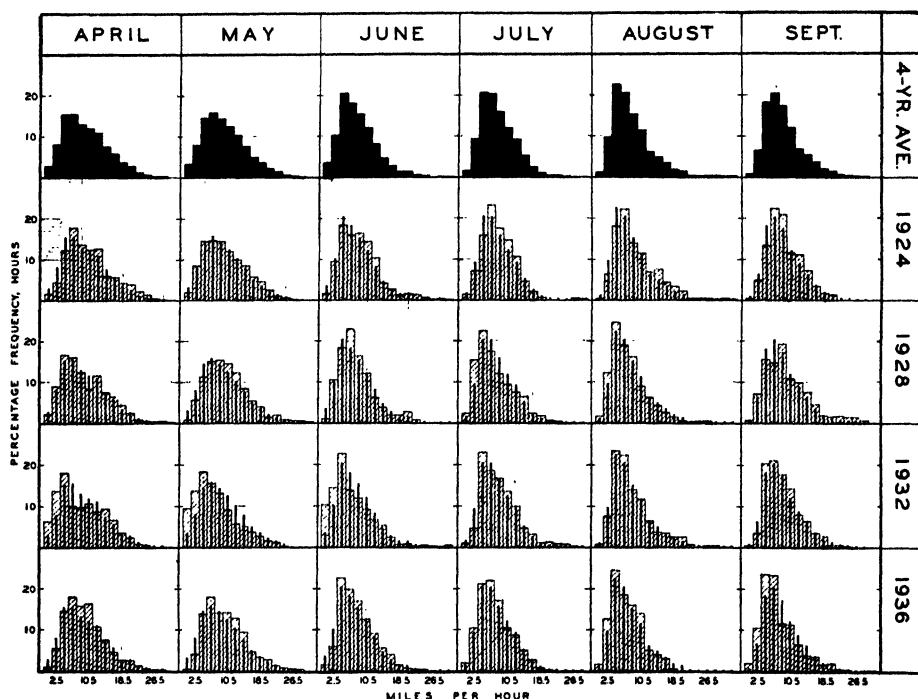
FIG. 5. *Percentage frequency distribution of hourly wind mileage at Qu'Appelle, Saskatchewan. Four-year average superimposed upon individual histograms by vertical bars.*

being superimposed on the individual histograms in the form of vertical bars to facilitate comparison. The general nature of the distribution is maintained from year to year, but there may be an appreciable fluctuation in the percentage of hours with specified mileages, particularly at the lower end of the range which, as has previously been suggested, is probably the region of most importance in its effect on evaporation and transpiration. As in the case of the daily mileages, the average proportion of lower values is significantly greater at Lacombe than at Qu'Appelle.

Relation between Daily Temperature and Wind Mileage

The final point to be considered was the nature of the association, if any, between daily temperature and wind mileage. Even a moderate degree of correlation between these two weather elements during the summer months

would, of course, be a decidedly adverse feature from the agricultural point of view. On the other hand, if they fluctuate independently, this circumstance must be taken into account. In order to study this point, daily mean temperatures at Qu'Appelle for the months of April, June and September were computed from the daily maxima and minima, and are shown plotted against the corresponding daily wind mileages in Fig. 7.
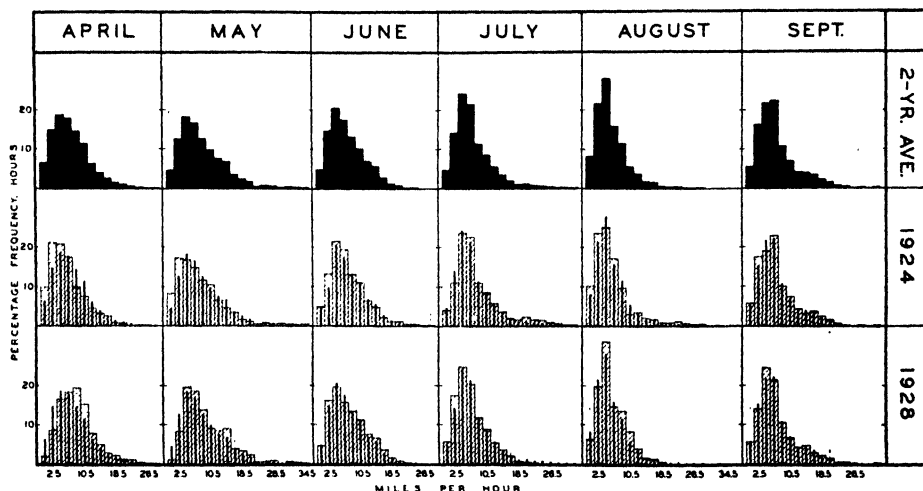
FIG. 6. *Percentage frequency distribution of hourly wind mileage at Lacombe, Alberta. Two-year average superimposed upon individual histograms by vertical bars.*
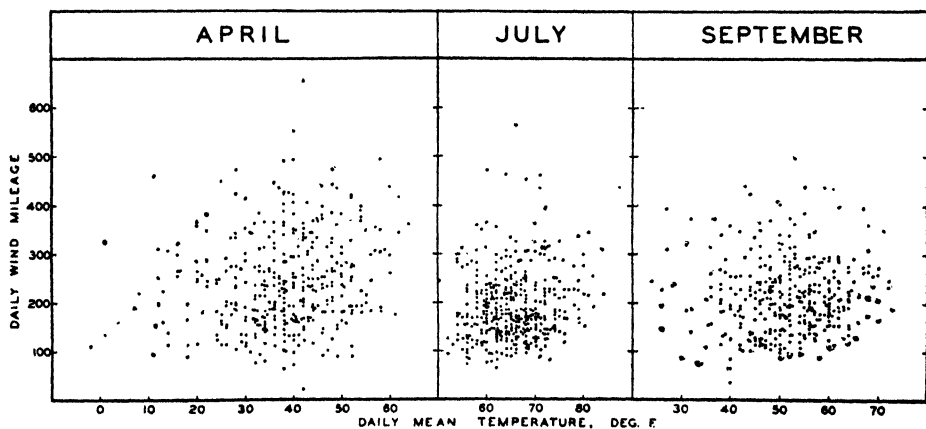
FIG. 7. *Daily temperature and wind mileage at Qu'Appelle, Saskatchewan, 1922–1937.*

The intra-monthly association of these two quantities yielded simple correlation coefficients of 0.147 for April, 0.141 for July and 0.014 for September. These are all small, but before concluding that there is little correlation between daily temperature and wind mileage, it is necessary to take

account of the fact that their average seasonal trend is dissimilar, monthly mean temperature being at its maximum in midsummer, when monthly wind mileage is at its minimum. This circumstance might operate to mask an actual positive intra-monthly correlation, but apparently did not in fact do so to any appreciable extent, as the correlation was not effectively altered by the elimination of the linear intra-monthly secular trend, the partial coefficients being 0.144, 0.141, and 0.058 for the three months respectively. As these are computed from between 300 and 400 daily values in each case, the first two, although small, are statistically significant, exceeding the 1% point. It may be surmised, therefore, that there is a slight tendency for days of above-average temperature to have a higher wind mileage, but that this is a minor factor in comparison with the uncorrelated variation of both quantities.

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VARIETAL DIFFERENCES IN BARLEYS AND MALTS

V. WORT NITROGEN AND MALT EXTRACT AND THEIR CORRELATIONS WITH BARLEY NITROGEN FRACTIONS[1]

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Abstract

Data representing 12 barley varieties grown at 12 experimental stations in Canada were used to examine relations among wort nitrogen (w), malt extract (e), barley nitrogen fractions (x), and total barley nitrogen (n).

Significant *inter-varietal* partial correlations, independent of total nitrogen, were found between wort nitrogen and salt-soluble nitrogen ($r_{wz.n} = 0.88$), and between extract and salt-soluble and insoluble nitrogen fractions ($r_{ez.n} = 0.60$ and -0.65). Partial correlations between wort nitrogen and alcohol-soluble and insoluble nitrogen were negative but insignificant ($r_{wz.n} = -0.54$ and -0.32).

Intra-varietal simple correlations between wort nitrogen and insoluble, alcohol-soluble, and salt-soluble nitrogen fractions were all positive and significant $(r_{wz} = 0.77, 0.74,$ and $0.61)$, and those between extract and each fraction were all negative and significant $(r_{ex} = -0.88, -0.96$ and $-0.77)$. All of the corresponding partial correlation coefficients were insignificant.

The differences between the inter- and intra-varietal relations can be summed up as follows: between varieties, total nitrogen and factors associated with it have little influence on wort nitrogen or extract, whereas nitrogen distribution and factors associated with it have a measurable effect; within varieties, total nitrogen and factors associated with it are largely responsible for the control of wort nitrogen and extract, and one of these factors is nitrogen distribution. The differences result mainly from the fact that whereas within varieties nitrogen distribution is closely related to total nitrogen content, between varieties nitrogen distribution is independent of total nitrogen content.

The relations between extract yield of malt and various barley properties have been investigated by Bishop (4–7). He made statistical studies of the relations of extract with total nitrogen, insoluble carbohydrates, 1000-kernel weight and percentage germination of the barley, and with wort nitrogen, and used these properties in developing prediction equations for malt extract. So far as the present authors are aware, no statistical study has been made of the relations between malt extract and barley nitrogen fractions, or of those between wort nitrogen and barley nitrogen fractions. It appeared that an investigation of these relations might yield useful information and it was accordingly undertaken.

Data and Methods

The experimental data were collected by analysis of 144 samples of barley and of the malts made from them. The samples represent 12 varieties grown at 12 widely separated experimental stations in Canada. A detailed description of the varieties and of the methods used in growing the samples was

published in Part I of this series (1), which also contains summaries of the data on the total nitrogen and nitrogen fractions of the barley samples. A description of the methods used for making and analyzing the malts, and summaries of the data on extract yield and wort nitrogen, were published in Part IV (9).

The relations between each pair of properties were examined by calculating (i) the simple correlation coefficient, and the partial correlation coefficient, independent of total nitrogen, for varietal means over all stations, which represent the average inter-varietal relation; and (ii) the simple and partial correlation coefficients for station means over all varieties, which represent the average intra-varietal relation. Some multiple correlation coefficients and regression equations were also calculated when it seemed probable that these statistics would prove illuminating.

Relations between Wort Nitrogen and Barley Nitrogen Fractions

Correlation coefficients showing the relations between wort nitrogen and barley nitrogen fractions, and wort nitrogen and total barley nitrogen are given in Table I. The independent variables are listed in the first column, and the relation between wort nitrogen and each of these is represented by a row of four correlation coefficients, namely, the simple and partial correlation coefficients for varieties and stations.

TABLE I

RELATIONS AMONG WORT NITROGEN (w), BARLEY NITROGEN FRACTIONS (x), AND TOTAL BARLEY NITROGEN (n)

x = independent variables listed below	Correlation coefficients			
	Varieties		Stations	
	Simple, r_{wx}	Partial, $r_{wx.n}$	Simple, r_{wx}	Partial, $r_{wx.n}$
Insoluble protein nitrogen	− .231	− .319	.769**	.253
Alcohol-soluble protein nitrogen	− .175	− .537	.736**	− .150
Total salt-soluble nitrogen	.887**	.885**	.610*	.118
Salt-soluble protein nitrogen	.827**	.824**	.564	.005
Non-protein nitrogen	.863**	.841**	.522	− .289
Total nitrogen	.166	—	.764**	—
Residual degrees of freedom	10	9	10	9

NOTE: *In this and later tables, ** denotes that the 1% level, and * that the 5% level of significance is attained.*

Inter-varietal Relations

The inter-varietal relations between wort nitrogen and the barley nitrogen fractions are represented by the simple and partial correlation coefficients given in the first two columns of data. In considering the latter it is useful to think of them as coefficients of correlation between wort nitrogen and the property in question, after both have been adjusted for varietal differences

in total nitrogen content, *i.e.*, after the complicating influence of varietal differences in total nitrogen content has been removed. Comparison of the partial and simple correlation coefficients shows that, with respect to the inter-varietal relations, this complicating influence is relatively small. It will be wise, nevertheless, to confine further discussion to the partial correlation coefficients.

These statistics show that between varieties wort nitrogen is directly correlated with total salt-soluble nitrogen and with the two fractions into which this was split, namely, salt-soluble protein nitrogen and non-protein nitrogen. A relation of this sort might well be expected on common-sense grounds. The wort is a dilute salt solution and should contain a very considerable proportion of the salt-soluble nitrogen compounds even if these were not hydrolyzed by proteolytic enzymes during the malting and mashing processes. Moreover, the least soluble portions of the salt-soluble fraction would presumably require only mild hydrolysis to bring them into solution in the wort. We might thus expect the salt-soluble nitrogen fraction of the barley to make a fairly large direct contribution to wort nitrogen, which would be enhanced by enzymatic hydrolysis of the least soluble compounds during malting, and offset in part by a comparatively small loss of soluble nitrogen transferred to the roots and synthesized to less soluble proteins in the acrospire.

In the authors' opinion the partial correlation coefficients are surprisingly high since other factors, specifically the activity of the proteases, must play a part in controlling wort nitrogen. In this connection it is interesting to note that it was shown in Part III of this series (2) that an inter-varietal correlation exists between total salt-soluble nitrogen and total saccharifying activity of the barley (r = 0.739). The possibility of a corresponding correlation between salt-soluble nitrogen and proteolytic activity is thus suggested, and if such an association exists it might tend to raise the coefficient of correlation between this barley nitrogen fraction and wort nitrogen. Whatever the explanation may be, it seems clear that varieties having a larger proportion of their nitrogen in salt-soluble form tend to yield higher percentages of wort nitrogen.

It is also interesting to note that although the partial correlation coefficients for wort nitrogen and the two least soluble nitrogen fractions are not significant they are at least negative. This was to be expected since as the salt-soluble fraction increases the salt-insoluble fraction decreases, so that if the former is directly correlated with wort nitrogen the latter should be inversely correlated with wort nitrogen. This can be demonstrated by treating the insoluble and alcohol-soluble nitrogen as one fraction, salt-insoluble nitrogen. The coefficient of partial correlation between wort nitrogen and salt-insoluble nitrogen, independent of total nitrogen, proved to be −0.923.

Intra-varietal Relations

The intra-varietal relations between wort nitrogen and the other properties are shown by the simple and partial correlation coefficients given in the last

two columns in Table I. These present an entirely different picture from the corresponding one for the inter-varietal relations. The simple correlation coefficients for wort nitrogen and the two least soluble nitrogen fractions are positive and significant, and all the partial correlation coefficients are insignificant.

The explanation of the differences between the inter- and intra-varietal relations appears to lie in the fact that, whereas between varieties the nitrogen distribution is independent of total nitrogen, within varieties the distribution is more or less dependent upon total nitrogen. Thus Bishop (3) has shown that within varieties, as the total nitrogen increases, the amounts of each of the nitrogen fractions also increase more or less regularly, although not at the same rates (see also Part I of this series (1)). It thus follows that as salt-soluble nitrogen increases, alcohol-soluble and insoluble nitrogen increase also. Accordingly if a direct inter-varietal correlation is found between wort nitrogen and salt-soluble nitrogen, in accordance with expectations, direct correlations should also exist between wort nitrogen and each of the less soluble fractions.

In calculating the partial correlation coefficients adjustments are made, to both wort nitrogen and the fraction in question, for the effect of differences between stations with respect to total nitrogen. After these adjustments are made there remain only the variations resulting from irregularities in the generally regular increase in each fraction with increasing total nitrogen content. These remaining variations are not large in themselves, nor are they large in comparison with the experimental errors involved in determining them. In consequence little opportunity exists for demonstrating the possible existence of intra-varietal partial correlations between wort nitrogen and barley nitrogen fractions, independent of total nitrogen.

It seems clear that within varieties the main factors controlling wort nitrogen are associated with total nitrogen rather than with nitrogen distribution among the protein fractions. These factors are probably connected with proteolytic activity, which may well be correlated with total nitrogen within varieties, but not between varieties. Relations of this sort between malt saccharifying activity and total nitrogen have already been demonstrated in an earlier paper in this series (2).

Relations between Malt Extract and Barley Nitrogen Fractions

Inter-varietal Relations

Simple and partial correlation coefficients showing the inter-varietal relations between malt extract and each of the five barley nitrogen fractions, between malt extract and total nitrogen, and between malt extract and wort nitrogen, are given in the first two columns of data in Table II. Comparison of the simple and partial correlation coefficients shows that inter-varietal differences in total nitrogen have only a slight tendency to mask the real relations between extract and the various nitrogen fractions.

The partial correlation coefficients show that between varieties extract is inversely correlated with insoluble protein nitrogen and directly correlated with total salt-soluble nitrogen and salt-soluble protein nitrogen, but that significant correlations do not exist between extract and alcohol-soluble, non-protein nitrogen, or wort nitrogen.

TABLE II

RELATIONS AMONG MALT EXTRACT (e), BARLEY NITROGEN FRACTIONS AND WORT NITROGEN (x),
AND TOTAL BARLEY NITROGEN (n)

x = independent variables listed below	Correlation coefficients			
	Varieties		Stations	
	Simple, r_{ex}	Partial, $r_{ex.n}$	Simple, r_{ex}	Partial. $r_{ex.n}$
Insoluble protein nitrogen	−.728**	−.648*	−.883**	.022
Alcohol-soluble protein nitrogen	−.200	.201	−.958**	−.319
Total salt-soluble nitrogen	.452	.604*	−.767**	.306
Salt-soluble protein nitrogen	.590*	.696*	−.641*	.321
Non-protein nitrogen	.138	.341	−.785**	.014
Total nitrogen	.380	—	−.957**	—
Wort nitrogen	.431	.542	−.716**	.073
Residual degrees of freedom	10	9	10	9

It seems best to deal first with the relation between extract and wort nitrogen. The partial correlation coefficient (0.542) fails to attain the value (0.602) required for the 5% level of significance. Nevertheless, the value is high enough to suggest that a significant partial correlation might be demonstrated by investigation of a larger number of varieties.

In the present investigation the maximum difference between varieties with respect to wort nitrogen calculated as protein was 2.0%, whereas the maximum difference in extract yield was 5.8% (see (9) Table I). Thus it appears that the amount of wort nitrogen a variety yields can have only a relatively small influence on the total yield of extract, and a close correlation between wort nitrogen and extract would hardly be expected.

Since the partial correlation between wort nitrogen and extract is not significant, the significant partial correlations between the salt-soluble nitrogen fractions and extract cannot be wholly explained by the direct contribution which these make to wort nitrogen. Some other factors must be involved, but these cannot be the result of proteolytic activity since this must also contribute only to wort nitrogen. The possibility that these factors represent the activity of various hydrolytic enzymes, other than proteases, suggests itself and receives some support from the previously noted fact that an inter-varietal association between saccharifying activity and total salt-soluble nitrogen has already been demonstrated (2).

Since the insoluble protein (glutelin) can contribute to extract only by means of hydrolysis by proteolytic enzymes, an inverse correlation between this

fraction and extract might be expected. However, since in the present study the maximum difference between varieties with respect to insoluble protein was less than 0.9% (see (1) Table III), whereas the maximum difference in extract was 5.8%, it again seems surprising that a significant correlation should be obtained. A possible explanation may be that varieties that contain larger amounts of insoluble protein also tend to contain larger amounts of the insoluble carbohydrates, lignin and cellulose.

TABLE III

INTER-STATION PREDICTION OF EXTRACT (E) FROM TOTAL NITROGEN (N) AND TOTAL SALT-SOLUBLE NITROGEN (S)

$$E = 84.4 - 6.3 N + 5.1 S$$

Station	Total nitrogen, %		Total salt-sol. N, %		Extract, %	
	Value	Multiplied by −6.3	Value	Multiplied by 5.1	Predicted	Found
Gilbert Plains	2.69	−16.95	.807	4.12	71.6	71.6
Nappan	1.54	− 9.70	.598	3.05	77.8	78.0
Difference	1.15	− 7.25	.209	1.07	− 6.2	−6.4

An alternative explanation also suggests itself. Bishop (5), in discussing intra-varietal relations between total nitrogen and extract, points out that the decrease in extract with increasing total nitrogen is considerably greater than would be expected if the decrease resulted merely from displacement of soluble carbohydrates by nitrogen compounds. He therefore suggests that the protein, in addition to replacing carbohydrates, "seals up" some of the remaining carbohydrates, thus making them less readily extractable. It seems possible that this sealing up, if it exists, may be largely a function of the glutelin protein which might thus have a greater effect on extract yield than would be expected from consideration of its quantity alone.

Whereas the partial correlation coefficient for insoluble nitrogen is negative and significant, that for the alcohol-soluble fraction (hordein) though insignificant, is positive. It accordingly seems reasonable to suppose that the alcohol-soluble fraction can have little effect in any sealing up of soluble carbohydrates which may exist. Bishop (8) has suggested that the alcohol-soluble protein and starch play similar roles in the grain, both acting as reserves. The possibility of some inter-varietal association between these two reserve compounds suggests itself as a possible explanation of the fact that the partial correlation coefficient for alcohol-soluble nitrogen and extract, though insignificant, is positive. On the whole, it appears more surprising that it is possible to demonstrate, with only 12 varieties, that significant correlations exist between extract and certain nitrogen fractions, than that significant correlations cannot be demonstrated between extract and other fractions.

Intra-varietal Relations

The intra-varietal relations between extract and barley nitrogen fractions, extract and total nitrogen, and extract and wort nitrogen, are shown by the simple and partial correlation coefficients given in the last two columns in Table II. The intra-varietal relations for extract are similar to, but the reverse of, the corresponding intra-varietal relations for wort nitrogen. All the simple correlation coefficients are significant and negative and all the partial correlation coefficients are insignificant.

The explanation of the differences between the inter- and intra-varietal relations for extract also appears to lie in the fact that, whereas between varieties the nitrogen distribution is independent of total nitrogen, within varieties the distribution is more or less dependent upon total nitrogen content. Within varieties the alcohol-soluble protein nitrogen and the insoluble protein nitrogen are closely correlated with total nitrogen content ($r = 0.985$ and 0.926, respectively) so that little opportunity exists for demonstrating partial correlations between these fractions and extract, independent of the effect of total nitrogen. It is quite clear, however, that as the total nitrogen increases, these two fractions increase also, and extract decreases.

It is also clear that within varieties, as total nitrogen increases, the amounts of the more soluble nitrogen fractions and of wort nitrogen also increase slightly (1), and it appears that these increases are associated with a decrease in extract. Further consideration will suggest, however, that in spite of the inverse associations, increasing amounts of the more soluble nitrogen fractions may add to extract yield. Thus within varieties and with an increase in total nitrogen, the loss of extract resulting from displacement or binding of potentially extractable carbohydrates may well be far greater than the possible gain in extract resulting from a comparatively small increase in the amount of salt-soluble nitrogen compounds entering the wort. Common sense suggests that this hypothesis is sound and it also receives some support from the fact that the partial correlation coefficients for the more soluble barley nitrogen fractions, though insignificant, are at least positive.

The possible quantitative aspects of this matter can be brought to light by calculating the regression equation for extract (E) on total nitrogen (N) and total salt-soluble nitrogen (S). This turns out to be:—

$$E = 84.4 - 6.3\,N + 5.1\,S.$$

Results obtained by using this equation are shown in Table III. The data for total nitrogen and total salt-soluble nitrogen are mean values over all varieties, and Nappan and Gilbert Plains are the stations at which total barley nitrogen was highest and lowest (see (1) Table I). It will be observed that an increase in total nitrogen content of 1.15% results in a predicted decrease in extract of 7.25%. On the other hand, the small increase in total salt-soluble nitrogen of 0.208% results in a predicted increase in extract of only 1.07%. The net decrease in extract is thus 6.2%.

This example must be considered as a hypothetical illustration of the possible simultaneous relation between extract, total nitrogen, and total salt-soluble nitrogen. Since the coefficient of partial correlation between extract and total salt-soluble nitrogen is not significant, the available data fail to prove that the corresponding partial regression coefficient is of the order of the calculated estimate, 5.1. It may even be negative, though this seems rather improbable.

The differences between the inter- and intra-varietal relations for extract can be summed up as follows: between varieties total nitrogen and factors associated with it have little influence on extract, whereas nitrogen distribution and factors associated with it have a measurable effect; within varieties total nitrogen and factors associated with it are largely responsible for the control of extract, and among these factors nitrogen distribution must be numbered. The differences result mainly from the fact that whereas within varieties nitrogen distribution is closely related to total nitrogen content, between varieties nitrogen distribution is independent of total nitrogen content.

Simultaneous Inter-varietal Relations among Malt Extract and the Nitrogen Fractions, Total Nitrogen, and 1000-kernel Weight of the Barley

The multiple correlation coefficients given in the last three columns of Table IV were calculated with the object of determining whether the inter-varietal prediction of extract from barley nitrogen fractions could be improved by introducing total nitrogen or 1000-kernel weight, or both, as additional independent variables. It was obviously useful to study further only the three nitrogen fractions which yielded significant partial correlations with extract, independent of total nitrogen content.

TABLE IV

SIMULTANEOUS INTER-VARIETAL RELATIONS AMONG MALT EXTRACT (e), BARLEY NITROGEN FRACTIONS (x), TOTAL BARLEY NITROGEN (n), AND 1000-KERNEL WEIGHT OF BARLEY (g)

x = independent variables listed below	Correlation coefficients			
	Simple, r_{ex}	Multiple, $R_{e.xn}$	Multiple, $R_{e.xg}$	Multiple, $R_{e.xng}$
Insoluble protein nitrogen	− .728**	.738*	.737*	—
Total salt-soluble nitrogen	.452	.676	.844**	.859*
Salt-soluble protein nitrogen	.590	.748*	.836**	.875**
Total nitrogen	.380	—	.470	—
Residual degrees of freedom	10	9	9	8

The multiple correlation coefficients for extract, a nitrogen fraction, and total nitrogen are given in the second column of data. Comparison of these with the corresponding simple correlation coefficients, given in the preceding column, shows that an appreciable improvement was obtained with respect

that the two-stage extraction method (5% potassium sulphate followed by hot 70% alcohol), used for determining insoluble protein nitrogen, precludes its ready use for prediction purposes. There is reason to believe, however, that the determination could be made using a single solvent, possibly 8% sodium salicylate solution (10).

Analyses of Variance

In order to determine whether the multiple correlation for extract and two independent variables could be considered significantly higher than the simple correlation between extract and the first variable, the inter-varietal variance for extract was analysed into portions accounted for by: (i) the first independent variable; (ii) the added effect of the second independent variable; and (iii) the residual variance. When the mean square for the added effect of the second variable proves to be significantly greater than the residual mean square, the multiple correlation coefficient can be considered significantly greater than the corresponding simple correlation coefficient.

The resulting mean squares for seven of these analyses are given in the first three sections of Table V. It is apparent that significantly higher multiple correlations were obtained only by combining 1000-kernel weight with either total salt-soluble nitrogen or salt-soluble protein nitrogen.

The last section of Table V gives the mean squares for corresponding analyses designed to determine whether the addition of a third independent variable raised the multiple correlation coefficient significantly. The statistics show that no significant increase was obtained.

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AN ATTEMPT TO HYBRIDIZE ANNUAL AND PERENNIAL AVENA SPECIES[1]

By L. P. V. Johnson[2] and H. A. McLennan[3]

Abstract

Cross pollination of annual with perennial Avena species did not result in the production of hybrid seeds, but certain specific combinations produced ovary stimulation.

Success in hybridizing annual Triticum and perennial Agropyron species (1, 2) and the resulting interest in the production of new, perennial forage crops prompted attempts to hybridize annual and perennial Avena species. The objective was the production of a perennial plant having the general foliage and seed characters of annual, cultivated oats. However, after two years' work the results, herein reported, are essentially negative.

Nine annual and eight perennial Avena species were used (see Table I). The annual species *A. sativa* and *A. byzantina* were represented by six and two varieties, respectively, while the perennial species *A. pratensis*, *A. planiculmis* and *A. pubescens* were represented by six, four and two forms, respectively.

Using the annual species as maternal material, a total of 2,914 florets were emasculated and cross-pollinated (1,652 in 1937 and 1,262 in 1938). A total of 56 crossing combinations, not distinguishing between strains, were attempted. In most cases the 1938 work repeated the combinations attempted in the previous year.

The annual (and biennial) species were started in the greenhouse in late winter and transplanted to the field in the spring in order that flowering might coincide with that of the perennial species (early mid-June). In 1937 about 300 florets on potted plants were emasculated and cross-pollinated in the greenhouse. The hybridization technique was to emasculate the florets of the annual plants about three days prior to anthesis, bag with glassine envelope, and pollinate with perennial species three days later. Further details on the general technique used are outlined in a previous report (3).

Table I presents data on the material used and the number of florets cross-pollinated in each crossing combination attempted. No hybrid seeds were

obtained from any of the cross pollinations made. In the case of *A. sativa* var. Early Ripe pollinated by *A. pratensis* 1422 and by *A. montana* 1430, a number of definitely stimulated ovaries were observed (Fig. 1). It is questionable whether this indicates closer phylogenetic affinities between these forms than between forms which produced no ovary stimulation upon cross pollination. Such stimulation is not considered to be the result of actual fertilization, but rather the result of activation by a chemical agent produced by the foreign pollen, probably in the course of germination.

TABLE I

THE NUMBER OF FLORETS OF ANNUAL SPECIES CROSS-POLLINATED BY PERENNIAL SPECIES

Female parent (annual Avena spp.)	Male parent (perennial Avena spp.)									
	A. elatior L. 1131	A. versicula Vill. 1420	A. pubescens L. 1421, 1435	A. pratensis L. 1422, 1426, 1427, 1429, 1433	A. pratensis L. var. Hertifolium, 1432	A. planiculmis Schrad. 1423, 1425, 1431, 1436	A. montana Vill. 1430	A. bisuni Asch. 1434	A. compressa Tleuff 1437	Totals
A. sativa L. var. Victory				34		303			76	413
A. sativa L. var. Gopher				61	83	48	29	15		236
A. sativa L. var. Selection 76	24	18	12	105	38	57	30	29		313
A. sativa L. var. Eagle						58				58
A. sativa L. var. Black Bountiful[2]						21				21
A. sativa L. var. Sixty Day	10	69	51	23	85	60	35	6		339
A. byzantina C. Koch						16				16
A. byzantina var. Early Ripe	12	23	66	80[3]	64	200	56[3]	11		512
A. nuda L.			8	26	7	124	23	29		217
A. fatua L.				6		29				35
A. sterilis L.				10		162				172
A. abyssinica Hochst.				9		87			33	129
A. strigosa Schreb.				42		101				143
A. wiestii Steudel		21		18	11	202		24		276
A. brevis Roth.				34						34
Totals	46	131	137	448	288	1468	173	114	109	2914

[1] *Numbers refer to strains within species or varieties.*
[2] *Winter variety (biennial).*
[3] *Definite stimulation of certain cross-pollinated ovaries.*

It is concluded that the annual and perennial Avena species in question are either not intercrossable or are intercrossable to a degree which is insufficient to produce positive results within the limits of the present work. Further attempts to intercross annual and perennial Avena species should, accordingly, be more extensive than the present investigation both in the number of combinations and in the number of cross-pollinated florets in each combination.
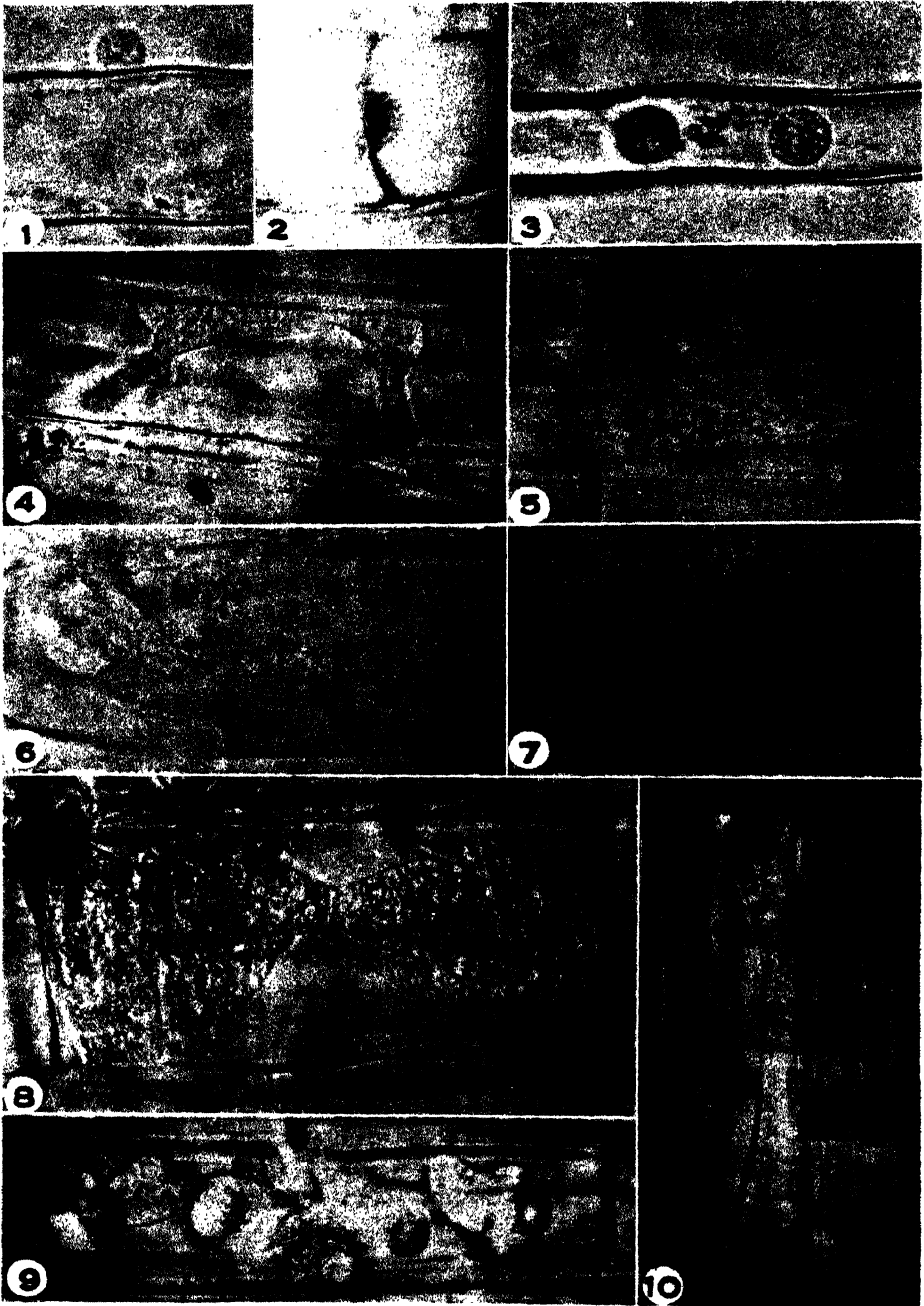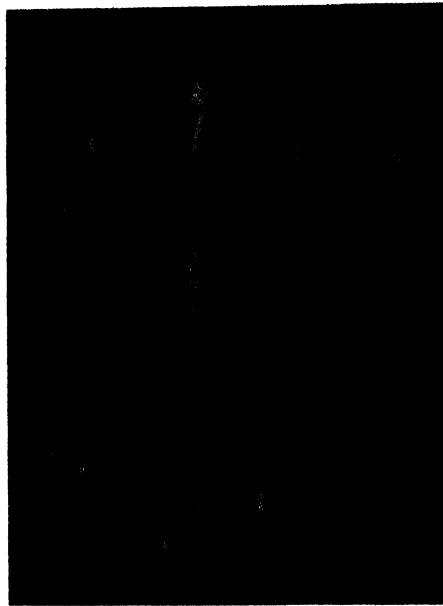
PLATE I

FIG. 1. *Left, normal seed. Upper right, unstimulated ovaries.*
Lower right, stimulated ovaries.

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STUDIES ON *POLYMYXA GRAMINIS*, N. GEN. N. SP., A PLASMODIOPHORACEOUS ROOT PARASITE OF WHEAT[1]

By G. A. Ledingham[2]

Abstract

A new member of the Plasmodiophorales with rather unusual characteristics has been found parasitizing the roots of wheat grown in soil from three different localities in Ontario. In addition to spore clusters of the *Ligniera* type, large, septate zoosporangia with conspicuous tubes for zoospore discharge are present. These multinucleate zoosporangia are produced by progressive lobular outgrowths from uninucleate amoebae and from the beginning are always surrounded by a thin wall. In the formation of resting spores, naked multinucleate myxamoebae develop first, then segment to form spore clusters without formation of a soral membrane. Both zoosporangia and resting spores produce identical zoospores with two flagella of unequal length.

The somatic nuclear divisions in the growing myxamoebae are characterized by the simultaneous division of both the nucleolus and chromatin within a persistent nuclear membrane. During the transitional phase which follows, the nucleolus disappears and at the same time there is an intensification of the staining properties of the surrounding cytoplasm. Prior to segmentation of the myxamoebae to form the spore clusters, and in all divisions during growth of the zoosporangia, the nucleoli and nuclear membranes disappear, and divisions are of ordinary mitotic type.

Relationship with the Plasmodiophorales is indicated by the form of the resting spore clusters, the method of nuclear division during growth of the myxamoebae, and the characteristic flagellation of the zoospore. Since the zoosporangial characteristics of this fungus differ from those of other genera within this order, it is considered desirable to place it in a new genus for which the binomial *Polymyxa graminis* is proposed.

Introduction

Apart from *Plasmodiophora brassicae* and *Spongospora subterranea*, which have been studied quite intensively on account of their world-wide economic importance, few species of the Plasmodiophorales have received much attention from plant pathologists. It was of interest therefore to find a member of this order present as an intracellular parasite in the roots of wheat, as no previous record of parasitism of cereals by the Plasmodiophorales could be found.

The fungus was first discovered in the autumn of 1929 during an investigation of certain root rots of wheat occurring in Ontario. During the following three years, studies were made on the organism in the Botany Department, University of Toronto, whenever infected roots were available. Later, special studies on germination of the resting spores and morphology of the zoospores

were made in the Laboratories of Cryptogamic Botany at Harvard University (7). In recent years most of the earlier observations have been rechecked and additional cytological studies have been made in the National Research Laboratories, Ottawa.

Resting spores were discovered first but it was soon found that large chytrid-like zoosporangia were associated with them in the wheat roots. It was thus of primary importance to determine whether these zoosporangia and resting spores belonged in the life cycle of the same organism. As a result of cultural and morphological studies there is now no doubt that this is so. The following reasons are given for this conclusion:

(1) Spore clusters and zoosporangia both developed in roots of wheat seedlings growing in nutrient solutions when inoculations were made with zoospores from zoosporangia only.

(2) Wheat plants grown in sterilized soil which had been inoculated with three-year-old, dried, finely powdered, infested roots produced both stages of the organism. Since it is very unlikely that the thin-walled zoosporangia can survive after drying, both types of thalli probably developed from germinating resting spores.

(3) The organism has been under observation for nine years and the two stages have invariably been associated on wheat from many different plantings in soil from different localities.

(4) During development, zoosporangia are most abundant at first, whereas the resting spores appear in greater numbers as the roots become more heavily infected. This would not be expected if the zoosporangia and resting spores were different organisms.

(5) Both spore clusters and zoosporangia produce identical zoospores of a characteristic biflagellate type which have been found to occur among the lower fungi only in the Plasmodiophorales.

As a result of the studies that have been made on this organism to date, the life history has been fairly well established. Such points as remain to be investigated are of a type on which information is still lacking on even the oldest members of the order. It seems advisable therefore to present the work as it now stands, leaving these questions for future consideration.

The absence in North American herbaria of all but a few species of the Plasmodiophorales, and the fragmentary accounts in the literature of some of the life histories, made it extremely difficult to determine the affinities of this new species. However, during a visit to Great Britain, the writer recently took advantage of the opportunity to compare it with most of the known species in all the genera of the order and was thus able to settle definitely the problem of relationship and nomenclature.

For reasons that will be discussed later it appears best to establish a new genus and species. Hereafter the binomial *Polymyxa graminis* will be used to designate this plasmodiophoraceous root parasite of wheat.

Material, Methods, and Host Relationships

The fungus was discovered in Marquis wheat roots that had been grown in soil obtained from the Central Experimental Farm at Ottawa. Later, wheat plants growing in the strawberry beds of the Vineland Horticultural Station were found to be infected, and soil from the Agricultural College Farm at Guelph also contained the organism. The heaviest infections of wheat were obtained in the greenhouse at about 60 or 65° F., using soil from Ottawa that had been frozen or dried for a few months.

Since there were no obvious external symptoms of disease in the host, such as hypertrophy or discoloration of the roots, microscopic observation was the only available method for detecting the presence of the fungus. Water mounts of living roots were quite satisfactory after thorough washing and removal of the air from the tracheids. However, the fungus stained so clearly with acid fuchsin or cotton blue in lacto-phenol that it was usually preferable to study stained material. If the roots were fixed in Bouin's or chrom-acetic fixer before lacto-phenol was added even nuclei were well preserved, but for fine cytological details microtome sections were superior. Difficulties due to hardening of the xylem in xylol were avoided by using an n-butyl alcohol series during the dehydration process.

Studies were made mainly with Marquis wheat as the host plant, but during the course of the investigation it was observed that Kubanka and Dawson's Golden Chaff wheat are also susceptible. In addition barley and rye, but not oats, have been infected. A similar type of resting spore was found in the roots of species of *Agropyron, Scolochloa, Rumex*, and *Impatiens*, but zoosporangia were not always associated with these. Several species of *Juncus* and *Poa* on which *Ligniera* had been reported in Europe did not become infected when grown along with parasitized wheat roots.

Morphology and Life History Studies

The Zoosporangia

The zoosporangium begins its development in the cortical cells of the wheat root as a uninucleate spherical thallus about three to four μ in diameter. The presence of a delicate surrounding membrane distinguishes it from the naked amoeba of similar size that will ultimately produce the resting spores. Nuclear divisions soon produce a multinucleate thallus, which may either remain spherical or become greatly elongated (Plate II, Fig. 14). The next step in the process is the production of a blunt lobular process from one side of this primordial thallus. A single nucleus moves out into this lobe and becomes much enlarged. Divisions follow and the new lobe becomes multinucleate and in turn sends out other lobes. In this manner a large septate thallus is developed which is completely surrounded from the beginning by a thin membrane (Plate I, Figs. 6, 7, 9). Although most of the lobate outgrowths are round or barrel-like in appearance, they may be very long and tubular, extending almost the entire length of the host cell. Development may then cease or a series of short blunt lobes may form at the end (Plate I,

Fig. 10). There are indications that a thallus of this type can pass through the walls of the host cell and invade neighbouring cells. A slight constriction occurs at the point of passage through the cell wall, and further development proceeds in the new cell.

The discharge tubes of the zoosporangium develop in the same manner as the lobes of the thallus. Short, blunt outgrowths grow toward the exterior wall of the host cell (Plate II, Figs. 11, 15 and 16). If the zoosporangium lies in an epidermal cell next to the surface of the root, a small round knob is formed which presses tightly against the cell wall. When the zoospores are formed a tiny opening appears in the centre of this knob through which they escape (Plate II, Fig. 20). Should the zoosporangium lie deeper in the cortex
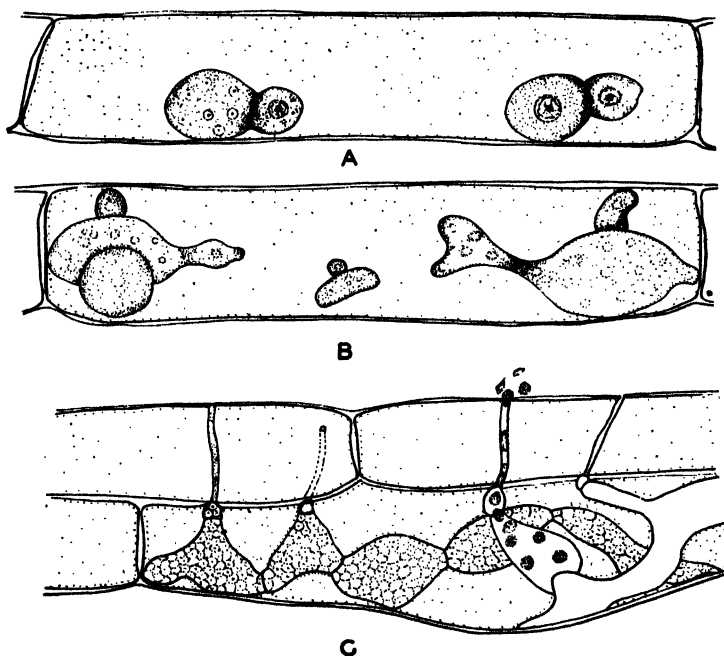

FIG. 1. *Polymyxa graminis.* FIG. 1A AND 1B. *Types of lobular outgrowths in early stages of zoosporangial development.* ×600. FIG. 1C. *Mature zoosporangia with discharge tubes passing through neighbouring host cells to reach the surface of the root.* ×600. *All figures drawn with the aid of the camera lucida.*

of the root, the discharge tubes may have to pass through adjacent cells to reach the exterior. A slight constriction appears at the point where the discharge tube passes through the cell wall and the tube remains long and narrow right to the surface of the root (Fig. 1C; Plate II, Fig. 19). Usually there is one discharge tube on each lobe of the zoosporangium, but occasionally there may be more (Plate II, Fig. 18). Viewed from the side the tube is cylindrical, and a septum is present at the point of connection with the zoosporangium, and also near the point of exit (Plate II, Fig. 16).

The question of whether the septa in the zoosporangium between the different lobes are true or pseudosepta can be determined most readily in zoosporangia that have discharged most of their zoospores. Zoospores trapped in the zoosporangium are confined to a single lobe and cannot pass through to another part of the thallus; also various lobes discharge their zoospores at different times. Therefore, the septum must be a true one. In the discharge tubes, however, openings occur in the septa to permit the passage of zoospores.

It is difficult to describe clearly all the changes which occur in the protoplasm of the thallus at the various stages of growth. Early in the course of development it is often finely granular and quite vacuolate in appearance (Plate I, Figs. 5 and 7). Some of the various changes in granularity, vacuolation and refringency of the protoplasm may be compared in the living thalli by examining Plate I, Figs. 3–10 and Plate II, Figs. 13, 15, 16, and 19. The fine refringent granules of a developing zoosporangium are clearly shown in Plate II, Fig. 15. The difference in appearance of a zoosporangium before and after zoospore delimitation is illustrated in Plate II, Fig. 13.

The walls of the empty zoosporangia tend to persist after the zoospores are discharged and later are frequently found in cells in which resting spore clusters have developed.
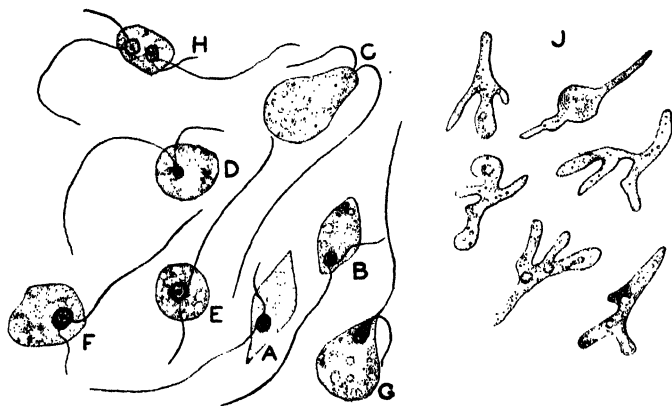

FIG. 2. *Polymyxa graminis.* A, B, C, D, E, AND F. *Zoospores illustrating some of the changes in shape.* × *1750.* FIG. 2H. *Binucleate spore with four flagella probably formed as a result of fusion of two uninucleate zoospores.* ×*1000.* FIG. 2J. *Amoeboid zoospores.* ×*1000. All figures drawn with the aid of the camera lucida.*

The Zoospores

The zoospores are fully developed within the zoosporangium and exhibit considerable movement even before they are discharged. Each zoospore oozes out of the end of the tube and appears at first as a small bubble on the surface of the host cell. Since there is no vesicle formed each zoospore is mature as soon as it leaves the discharge tube. When first released the zoospore is bulged in the centre with pointed ends (Fig. 2, A and B) and appears to be amoeboid for a moment; then the flagella begin to lash around and it
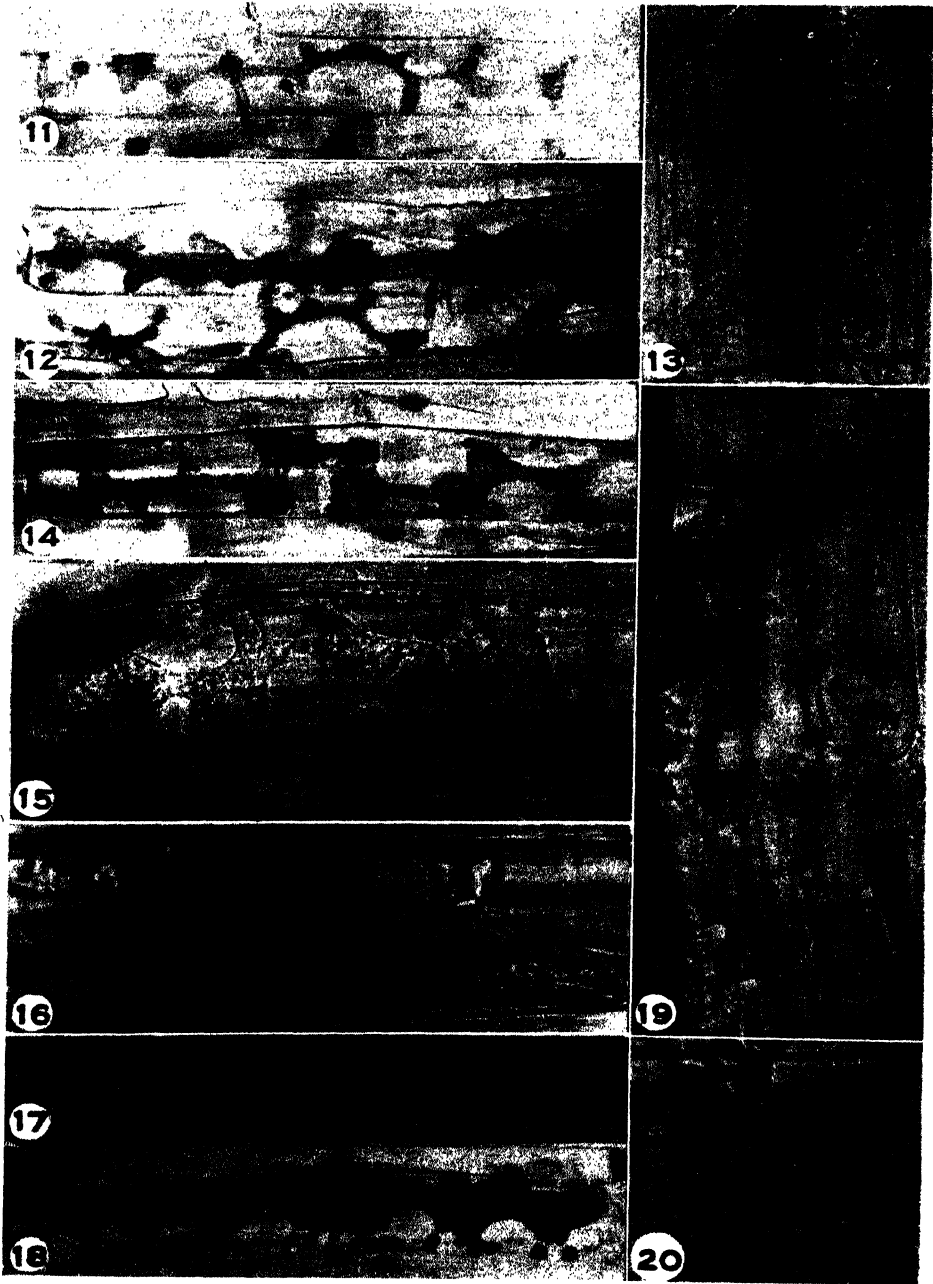
PLATE II

swims away with a rolling, turning motion. Should the zoosporangium lie deep in the cortex the zoospores will have to traverse the long slender discharge tube to the exterior (Fig. 1C). In such circumstances the zoospores pass along singly as in the mycelium of *Aphanomyces*.

From the work of previous investigators of the Plasmodiophorales, it was to be expected that the zoospores would be uniflagellate. Such appeared to be the case on first examination of living spores. However, it was noticed that even with the flagellum trailing, the zoospore was able to move rapidly, and the agitation among the bacteria and particles in the water at the anterior end of the spore close to the base of the long flagellum indicated that something was moving in that region. When stained preparations were made, proof was obtained of the presence of a short flagellum of about one quarter the length of the long one.

Most students of the lower fungi are agreed that zoospore flagellation is a very important character, which remains constant within a particular group. It was surprising, therefore, to fine one member of the Plasmodiophorales differing from the others in this respect. Accordingly, a re-investigation of the zoospores from the resting spores of *Plasmodiophora brassicae* and *Spongospora subterranea* was made, and they, too, were found to be biflagellate with a long and a short flagellum (8). The same character was found in the zoospores from the zoosporangia of *Spongospora subterranea* when these were discovered in 1935 (9), and just recently also for zoospores from spore balls of *Sorosphaera Veronicae*. This type of flagellation is quite different from that of any of the lower fungi or Myxomycetes and is not unlike that of heterokont algae (15). It is interesting to note that the short flagellum was also overlooked for many years in the algae, and its discovery caused a re-classification of the Chlorophyceae.

The living zoospore may be studied most conveniently in a very thin film of water on a cover slip over a Van Tiegham cell. The speed of swimming is thereby greatly reduced, making it possible to observe flagella, vacuoles and even the living nucleus. Under this very unfavourable environment it is not long before the zoospores encyst or become amoeboid. The period of zoospore activity depends largely on the environment, temperature being one of the most important factors. At 28° C. many zoospores become amoeboid and encyst in a few minutes, whereas at 18° C. they swim actively for several hours. If the zoospore is not in rapid motion it is generally spherical in shape. At times it may be top-shaped, pear-shaped, or ovoid (Fig. 2, A–E). Zoospores have been observed swimming with the flagella in front of the spore but usually they are propelled from behind. The body of the zoospore rotates over and over when in motion. If it is not very active it may rotate in a circle, pivoting around the long flagellum, which moves with a slow undulating movement, even when the zoospore is motionless.

After the zoospores have been swimming two or three hours under favourable conditions, or much sooner if the temperature is high, most of them become amoeboid. The flagella disappear and the amoebae move on the

surface of the substratum by means of pseudopodia (Fig. 2J).   These amoebae
vary greatly in shape and size.   Usually they are very vacuolate, and the
protoplasm in the pseudopodia has been observed to flow around and engulf
small objects.· It may be possible that they can ingest solid food particles
in this way, in the same manner as that described by Maire and Tison (10)
for zoospores of *Ligniera junci*.

Zoospores that have been fixed over osmic acid fumes, dried on a slide and
stained, are spherical in shape.   The average diameter of 150 stained zoospores
was 4.2μ, with a minimum of 2.5 and a maximum of 5.6μ.   The long flagella
are 16 to 20μ in length and the short ones 4 to 5μ.   A stained zoospore has
finely vacuolate cytoplasm (Plate III, Fig. 24) with dark granules scattered
throughout.   Frequently a large dark spot in the centre of a clear area is
visible on the margin of the zoospore.   This may be a vacuole.   The nucleolus
of a zoospore nucleus is not as conspicuous as that of the amoeba within the
host cell.   The nucleus is usually ovoid (Plate III, Fig. 24), but may occasion-
ally be top-shaped (Fig. 2G) or perfectly spherical (Fig. 2, E).

The flagella may be traced to the surface of the nucleus in stained material.
They have sometimes been found to be attached close together at the base
(Fig. 2D), though more often they are separated (Fig. 2, E and F).   Although
they appear occasionally to arise from the opposite poles of the nucleus, it is
believed in these instances that the densely stained nucleus may mask the
true proximity of the points of origin.   Occasional zoospores with four flagella
have been found (Plate III, Fig. 27).   Such spores are invariably binucleate
(Plate III, Fig. 28).   It has not been possible to determine whether they
are the product of a fusion between two zoospores or the result of incom-
plete separation within the zoosporangium.

The process of penetration by a zoospore has not been observed, although
many attempts have been made to do so by transferring zoospores to young
wheat rootlets growing in water or nutrient solution.   One zoospore which
had become amoeboid on the surface of a root hair was watched for 12 hours,
but it did not penetrate (Plate I, Fig. 1).   During this time several others
had passed into nearby root hairs and assumed a spindle-shaped amoeboid
form inside the host cell.   No empty membrane could be found on the surface
of the root hair after penetration of the zoospore as is the case with *Olpidium*.
It may, therefore, be assumed that the entire amoeba passes into the cell as
described by Curtis '(5) for *Synchytrium*.

The Resting Spore Clusters

The resting spore thallus begins development in the host cell as a naked
amoeba, and at no stage of development is a universal membrane present.·
Repeated nuclear divisions occur and a multinucleate myxamoeba is finally
formed.   Its spread being uncontrolled within the host cell, this myxamoeba
may assume many different forms.   Frequently it is long ·and tenuous, ex-
tending the whole length of the cell in which it started growth (Plate III,
Fig. 30), or it may form a crescent-shaped mass about the host nucleus.   At
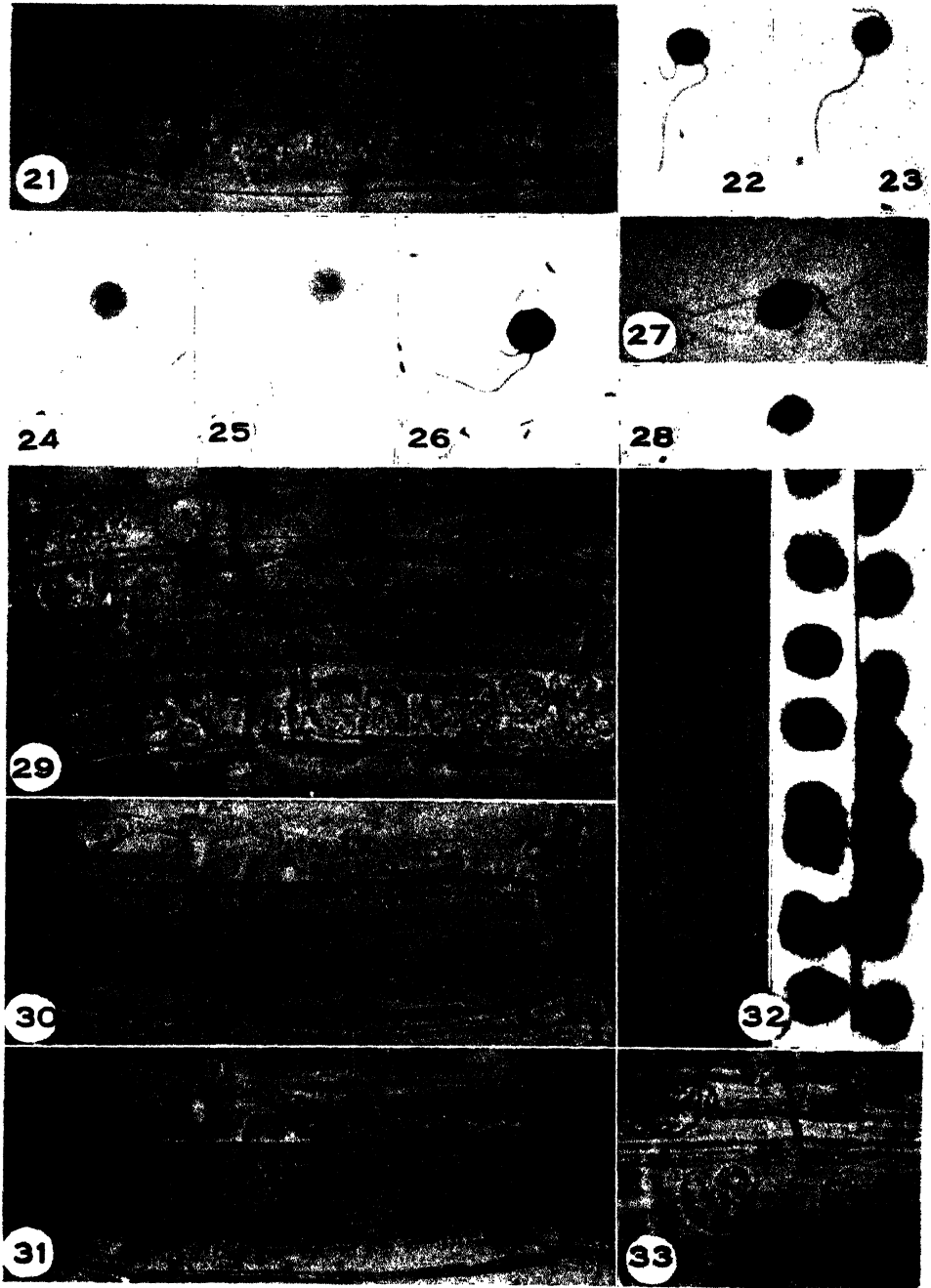
PLATE III

PLATE III. *Polymyxa graminis.* FIG. 21. *Mature zoosporangium with zoospores.* X266. FIGS. 22–25. *Zoospores from zoosporangia, stained.* X1200 FIG. 26. *Zoospore from resting spore, stained.* X1366. FIG. 27. *Binucleate spore with four flagella, stained.* X1200. FIG. 28. *Same spore as Fig. 27, showing nuclei, stained.* X1200. FIG. 29. *Zoosporangium; also meronts that will form resting spores.* X455. FIG. 30. *Naked myxamoebae, living.* X455. FIG. 31. *The same thallus as in Fig. 30, but 20 min. later.* X455. FIG. 32. *Meronts lying in tracheal cells: left, living; right, stained in lacto-phenol acid fuchsin.* X455. FIG. 33. *Same stage as in Fig. 32 but in cortical root cell.* X455.

this stage, long thread-like pseudopodia frequently radiate in various directions. Later these pseudopodia are retracted, the protoplasm of the fungus becomes much denser and the thallus may split up into a group of daughter cells or meronts, as termed by some writers. These meronts lie in rows or in closely packed groups in the tracheal and cortical cells (Plate III, Figs. 29, 32 and 33; Fig. 3, B and D). Fusions in the living fungus were occasionally observed among separate thalli lying in the same cell. Whether these were thalli of opposite sex or merely meronts from the division of a single mother
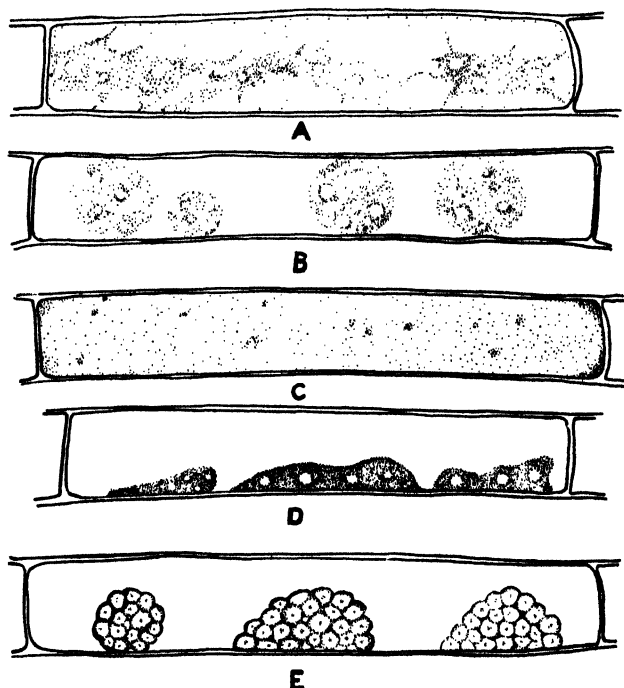
FIG. 3. *Polymyxa graminis.* FIG. 3A. *Naked myxamoebae during period of active growth.* ×600. FIG. 3B. *Meronts formed by division of myxamoebae.* ×600. FIG. 3C. *Same cell as in 3B, after the different meronts have coalesced.* ×600. FIG. 3D. *Myxamoebae just prior to the beginning of segmentation.* ×600. FIG. 3E. *Segmentation of myxamoebae to form resting spore clusters.* ×600.

thallus was not known. In one instance the granular protoplasm of four thalli kept milling slowly around until suddenly their contents spread out and fused into one mass, which filled the lumen of the host cell (Fig. 3, B and C). In another instance an elongate mass of naked protoplasm (Plate III, Fig. 30) suddenly spread out evenly in the host cell (Plate III, Fig. 31). The reason for the above phenomenon is not clear at present but is mentioned because it may have some significance in nuclear fusion.

During early stages of growth the myxamoeba appears very vacuolate, but as further development takes place the vacuoles gradually decrease in size and the thallus finally becomes a closely packed mass of dull granules.

(Plate III, Figs. 29 and 33). The density increases further and oily substances appear, which increase the refringency of the thallus just prior to spore delimitation (Plate IV, Fig. 39). Lines of cleavage appear around each nucleus, and by degrees cell walls are laid down, each consisting at maturity of an inner hyaline layer and an outer, smooth, brownish-yellow wall, which is fused with the neighbouring cells (Plate IV, Figs. 37 and 38). Most of the cells are quite spherical, but pressure exerted on the sides may cause some to be flattened or many-sided (Plate IV, Figs. 37 and 40). Individual cells of the spore clusters measure from 5 to 7μ in diameter. The contents are smooth and refringent without conspicuous oil droplets (Plate IV, Fig. 38).

It scarcely seems necessary to analyse the shape and size of the spore clusters in relation to those reported in the other genera as Palm and Burke (13) have done for *Sorosphaera Veronicae*, but some comparisons may be useful. Most of the spores are similar to those reported for *Ligniera junci* by Cook (1). Occasionally, individual spores similar to those of *Plasmodiophora brassicae* may be found (Plate IV, Fig. 36). Four spores grouped together as in *Tetramyxa* have been observed, and other clusters sometimes resemble the spore cakes of *Sorodiscus*, except for the absence of the universal membrane. The size and shape of the spore clusters and the great numbers present in heavily infected roots are shown in Plate IV, Figs. 35 and 36. Practically every cortical cell and even some of the tracheids in such roots are filled with spore clusters. Sometimes longitudinal rows of cells are infected (Plate IV, Fig. 34). This indicates that the fungus has been transferred from cell to cell during multiplication by division of an original infected meristematic host cell. There is no evidence that the myxamoeba can pass from cell to cell in non-meristematic host tissue.

Germination of the spores has been obtained on several occasions, but only after a prolonged treatment of alternate wetting, drying, and freezing. When zoospores appeared in a hanging drop of water containing a number of spore clusters, it was apparent from comparison of number and diameter of zoospores with empty cells in the spore cluster, that only one zoospore was produced by each cell. In manner of swimming and type of flagellation these zoospores were identical with those from the zoosporangia. Other writers have used the term swarm cells or swarm spores for these zoospores, as this is the terminology used in the Myxomycetes, but it seems preferable to discontinue this usage now, as the zoospores of the Plasmodiophorales are quite different from those of the Myxomycetes.

Cytological Studies

In all the species of Plasmodiophorales studied cytologically, nuclear behaviour during growth of the myxamoeba has been found to differ from ordinary mitosis. During the period of vegetative growth of the myxamoeba the conspicuous nucleolus persists and divides simultaneously with the chromatin within the nuclear membrane. On account of the unusual configurations, which at the metaphase take the form of a cross, it has sometimes

PLATE IV

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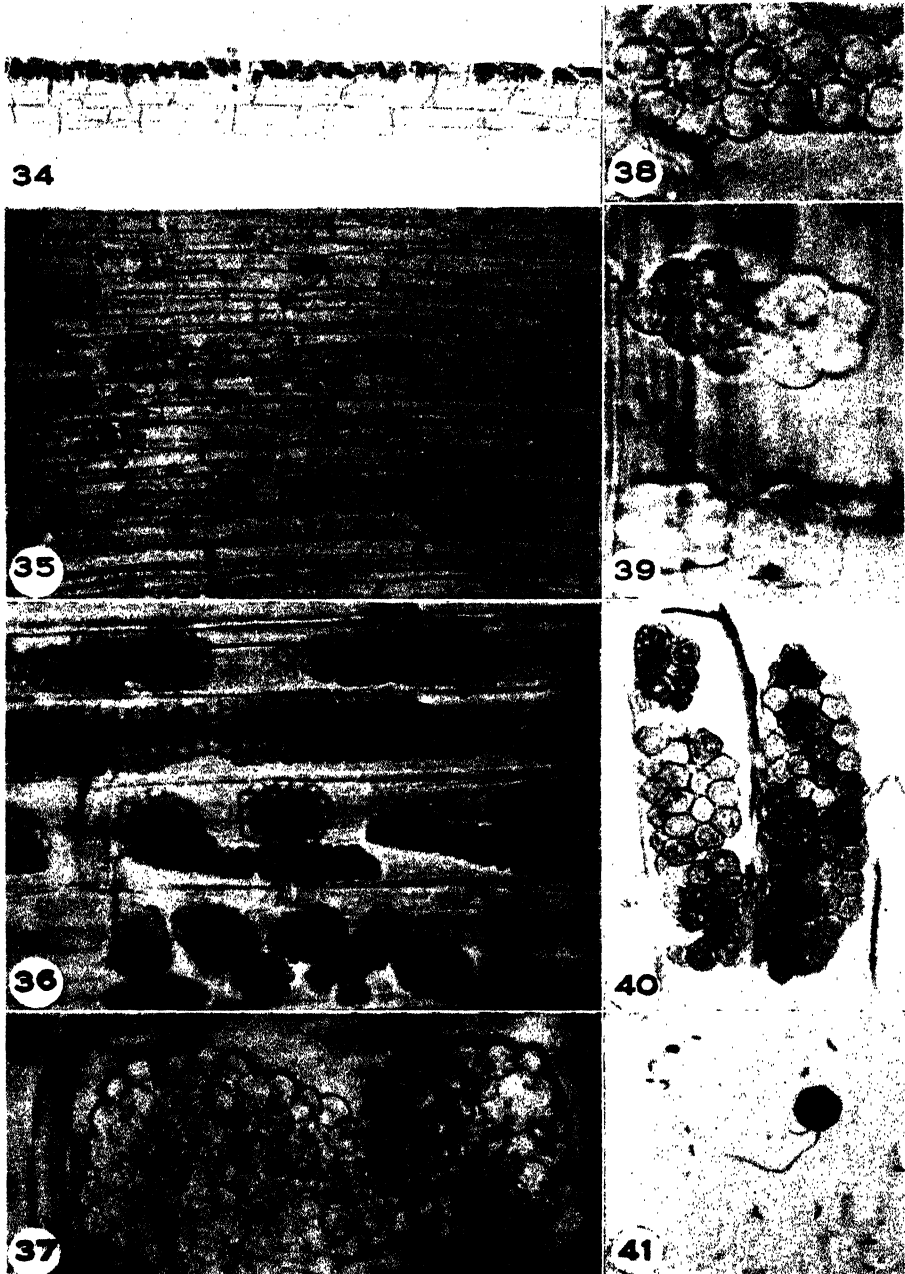
PLATE IV. *Polymyxa graminis.* FIG. 34. *Living spore clusters lying in one row of epidermal cells.* X90.
FIG. 35. *Strip of epidermal root tissue showing density of infection and varied shapes of spore clusters, stained,*
lacto-phenol acid fuchsin. X405. FIG. 36. *Spore clusters, stained lacto-phenol acid fuchsin.* X468. FIG. 37. *Two*
characteristic spore clusters, living. X925. FIG. 38. *Individual cells of a spore cluster showing thickness of wall,*
living. X1366. FIG. 39. *Stage in development of spore cluster, walls just being formed, living.* X1366. FIG. 40.
Section through spore clusters, stained safranin gentian-violet. X1200. FIG. 41. *Zoospore produced on germination,*
stained. X1200.

been called "cruciform division" but is more commonly known as proto-mitosis. Since this phenomenon has been observed frequently in the Plasmo-diophorales and not elsewhere among the fungi, Cook (2) has suggested that the presence of protomitosis might be used as a diagnostic character of the group. Although it is not proposed to present a detailed account of the cytology of the fungus described in this paper, sufficient evidence will be presented to show that it is not unlike other members of the order in its nuclear behaviour.

The Somatic Nuclear Divisions in the Myxamoebae

The first indication of nuclear division in the myxamoeba is an enlarge-ment of the nucleus and an increase in the intensity of staining of the chromatin granules, which radiate out from the nucleolus like the spokes of a wheel. Gradually a ring of chromatin appears and in polar view it completely sur-rounds the nucleolus. At right angles to the polar axis the ring appears as a band across the nucleolus, but if viewed at a slightly different angle the latter may lie slightly above or below it (Plate V, Figs. 45 and 48). This con-figuration is similar to that called the "balance scale" by Nawaschin (11), or the "unipolar spindle configuration" by Horne (6). No asters or centro-somes were found at this stage as reported by Nawaschin in his studies. The nucleolus now elongates and projects through the ring of chromatin as a short blunt peg. Further elongation of the nucleolus produces the typical "cruciform" figures which have so often been described by the various workers on the cytology of the Plasmodiophorales (Plate V, Figs. 44–48). Apart from the presence of the nuclear membrane and the division of the nucleolus with the chromatin, this is in reality a ring-like metaphase figure and has been described as such by Horne (6). The chromatin was distinctly granular in all rings examined, and in some cases was organized into more or less definite bodies believed to be chromosomes. The next step in division involves splitting of the ring into two halves that move apart toward the ends of the cylindrical nucleolus. Horne (6) calls this the telophase. It corresponds to the "double anchor" or "dumb-bell" figures of other writers (Plate V, Figs. 49 and 50). Next the two halves of the nucleolus contract and separate com-pletely, the spindle fibres disappear, and the nuclear membrane, which has been present throughout the whole process of division, constricts and closes in around the daughter nuclei. Thus through repeated divisions of this type a multinucleate thallus is developed. There is remarkable uniformity within a single myxamoeba in the time of occurrence of the different phases of nuclear division.

The Transitional Phase

The nucleoli disappear when the myxamoeba has attained its full growth, and simultaneously densely staining granules appear in the cytoplasm sur-rounding the nuclei. This has been called the akaryote stage by Cook (2), Osborne (12), and Schwartz (14), and the transitional phase by Horne (6) and Webb (17). Although earlier students thought the chromatin was

also extruded, leaving an empty nucleus, Webb (17) has demonstrated that this is not true, because a fine chromatin reticulum may be seen within the nuclear membrane if Newton's iodine-gentian-violet method of staining is employed. Webb's observation has been substantiated in the present study. In preparations stained with iron-alum haematoxylin the nuclei appeared to be devoid of chromatin, whereas iodine-gentian-violet revealed its presence. In *Sorosphaera Veronicae*, Webb has described a nuclear fusion during this phase; but although nuclei have been found associated in pairs, it was impossible in the present study to determine whether actual fusion took place.

Nuclear Divisions Preceding Spore Formation

By analogy with other species of Plasmodiophorales, meiosis should occur in the divisions that precede the segmentation of the myxamoebae into the spores that make up the spore clusters. So far it has been impossible to count the number of chromosomes present in the nucleus, so definite proof of meiosis must await further studies. In division figures found at this stage of development of the fungus, both the nucleolus and the nuclear membrane had disappeared, and the chromatin formed prophase, metaphase, anaphase, and telophase configurations (Plate V, Figs. 52–57) of the ordinary type found in higher plants.

Nuclear Divisions in the Zoosporangia

Throughout the whole period of growth of the zoosporangium there is no division of the nucleolus with the chromatin within the nuclear membrane comparable to that observed in the growing myxamoeba. All divisions were of the ordinary mitotic type, and even in the so-called resting nucleus no conspicuous nucleolus was present. These observations are in agreement with those made by Cook and Schwartz (4) on the nuclear divisions in the zoosporangia of *Plasmodiophora brassicae*, except that they state that the resting nucleus resembles in all essential features the resting nucleus of the plasmodium. In *Ligniera junci*, however, Cook has described protomitotic nuclear division in early stages of zoosporangial formation.

Discussion of Systematic Position

In considering the affinities of *Polymyxa graminis* with other non-filamentous intracellular parasites, it is obvious that the character of the resting spores alone separates it from the Synchytriaceae, Woroninaceae, Olpidiaceae and Ancylistaceae. Superficially the zoosporangia bear some resemblance to those of *Septolpidium lineare*, described by Sparrow (16) in 1933, since their course of development is similar, and at maturity they consist of a series of conjoined truncated segments. However, the fact that *Septolpidium* is an algal parasite with uniflagellate zoospores, which form a vesicle when discharged through non-septate discharge tubes, indicates at once that it is not closely related to *Polymyxa*.

Several lines of evidence, such as the type of resting spore clusters that develop from multinucleate myxamoebae, the method of nuclear division during
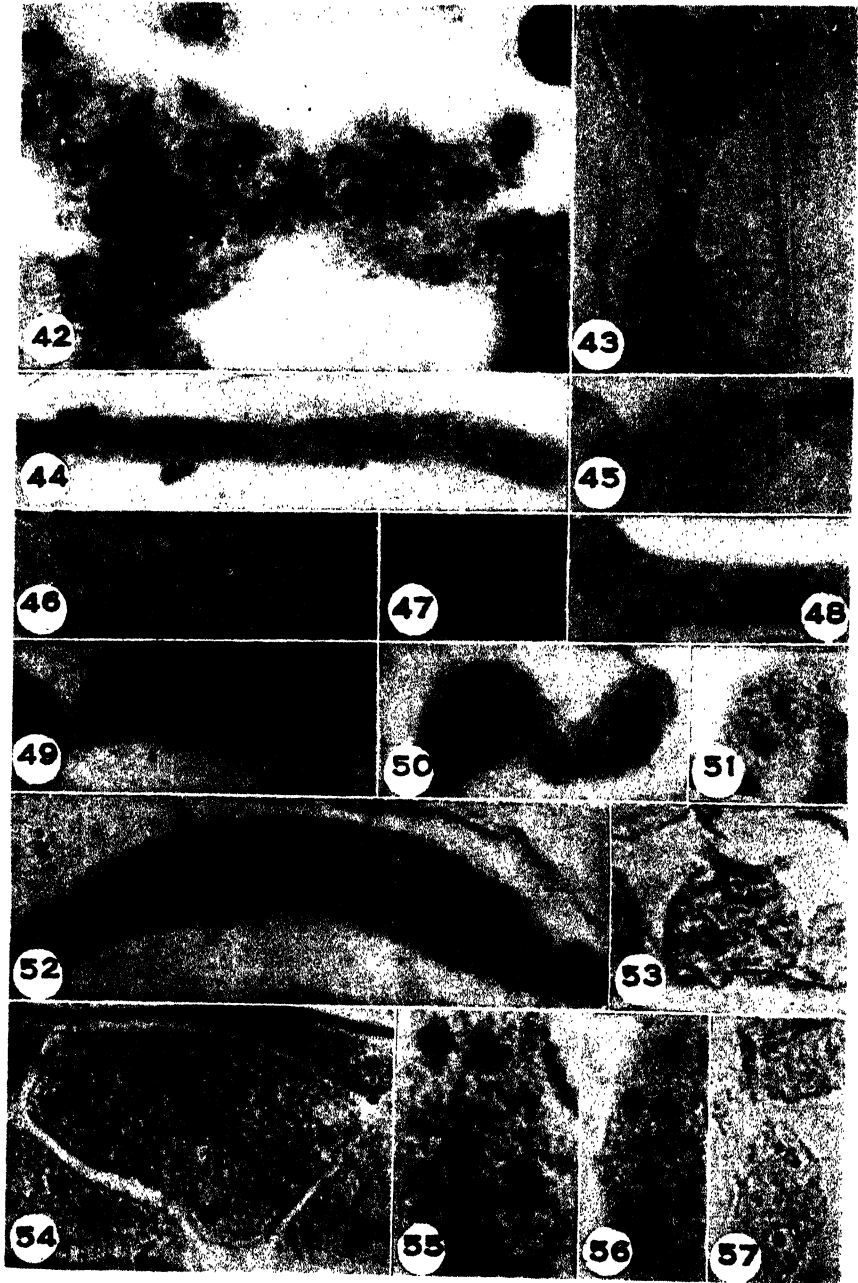
PLATE V

growth of the myxamoeba, and the characteristic biflagellate zoospores, point to relationship with the Plasmodiophorales. Generic differences in this order have hitherto been made almost entirely on the arrangement of the mature resting spores. The presence of spore clusters in *Polymyxa* separate it from *Plasmodiophora*, in which the spores are free. Inclusion with either *Sorodiscus* or *Sorosphaera* is not justified, because the spores in these genera are grouped into flat spore cakes or spore balls which are surrounded by a universal membrane. Likewise the spongy spore balls of *Spongospora* or the tetrad-like groups of spores in the genus *Tetramyxa* are quite different. Only with *Ligniera*, in which the spores are arranged in aggregates of indefinite size or shape, is there marked similarity of resting spore characters, but the dissimilar zoosporangia and absence of schizogony in this genus preclude the inclusion of *Polymyxa*.

Until quite recently zoosporangia were not thought to be present in the Plasmodiophorales. Cook (1) was the first to describe them in *Ligniera junci*, followed by Cook and Schwartz (4) for *Plasmodiophora brassicae*, and the writer (9) for *Spongospora subterranea*. In these three species the zoosporangia are small simple structures lacking zoospore discharge tubes. In *P. brassicae* and *S. subterranea* they are very evanescent bodies usually present in the root hairs of the host. Although these zoosporangia appear to be quite different from the conspicuous, highly organized type present in *Polymyxa*, when direct comparisons were made with *Spongospora subterranea*, the zoospores produced from each were found to be almost identical. In fact it is the presence on such zoospores of two flagella, one long, the other short, that provides the best evidence for close relationship between these species, even though there is considerable difference between the two species in zoosporangial and resting spore characters. In this respect the Plasmodiophorales are by no means unique, for it is a well known fact that within the different families of lower fungi, zoospore characters are quite similar in different species and genera of a single family, whereas there is frequently considerable variation in the zoosporangia and resting spores. The practice has generally been to use these latter differences for the separation of genera and species. Thus in the Olpidiaceae such characters as the location of the sporangia in the host cells, the presence or absence of exit tubes on the zoosporangia, and companion cells on the resting spores, are of importance. Indeed, the zoosporangia provide most of the important characters used to separate genera in several families of the lower fungi. There is ample precedence therefore to place more emphasis on zoosporangia in classification of the Plasmodiophoraceae. Most of the present difficulties in the taxonomy of this family are due to lack of knowledge of complete life cycles, thus making it necessary to place too much emphasis on minor differences in resting spores as a means of separating genera. Palm and Burke (13) have even suggested that it might be preferable to merge all the present genera—with the exception of *Cystospora*, which is a doubtful member—into the genus *Plasmodiophora*. It seems to the writer that this proposal only transfers the difficulties

encountered in separating genera to the species, without improving the present system of classification. For the present it appears advisable to retain the six genera recognized as valid by Cook (3) in his monograph of the Plasmo-diophorales, adding to these the new genus *Polymyxa* with the following diagnosis.

Diagnosis

Polymyxa n. gen.

Resting spore clusters of indefinite size and shape, without universal mem-brane; produced by schizogony and segmentation from naked multinucleate myxamoebae. Zoosporangia conjoined series of thin-walled, lobular, multi-nucleate segments, each with one or more discharge tubes. Similar zoospores produced by resting spores and zoosporangia, each bearing two flagella, one long and one short.

Polymyxa graminis n. sp.

Resting spores spherical or many sided, 5 to 7μ in diameter, smooth, yellow-brown outer walls. Zoosporangia large, septate, with persistent, smooth, thin walls. Discharge tubes segmented. Zoospores numerous, 4 to 5μ in diameter, discharged without vesicle formation. Non-hypertrophying, obli-gate parasite, in roots of *Triticum aestivum* L., *T. durum* Desf., *Hordeum vulgare* L., and *Secale cereale* L.

Type specimens are deposited in the herbarium of the Botany Department, University of Toronto, Ontario.

Polymyxa genus novum

Glomerulis sporarum quiescentium magnitudine et forma indefinitis, sine membrano universali, per fissionem sejunctionemque e nudis multinucleatis myxamoebis generatis. Zoosporangiis in seriem tenui-tunicatorum, lobula-torum, multinucleatorum segmentorum conjunctis, germinatione zoosporas per unum vel plures processus evacuantibus. Zoosporae e sporis quiescentibus formatae sunt similes illis e zoosporangiis exeuntibus; singulae zoosporae duo flagella, unum longum, sed alterum breve, ferent.

Polymyxa graminis species nova

Sporis quiescentibus globosis vel multilateralibus, levibus, fulvis, 5 to 7μ diam. Zoosporangiis magnis, septatis, cum muris persistentibus levibus tenuibus. Processibus evacuationis in segmenta divisis. Zoosporis copiosis, 4 to 5μ diam., sine origine vesicarum emissis. Thallo parasitica obligato, non tumefaciente in radicibus matricis.

In radicibus Tritici aestivi L., T. duri Desf., Hordei vulgaris L., et Secalis cerealis L.

Acknowledgments

Grateful acknowledgment for helpful suggestions is also made to Professor W. H. Weston of the Laboratories of Cryptogamic Botany, Harvard University, Professor B. Barnes of Chelsea Polytechnic, London, Professor W. R. I. Cook of the University of South Wales and Monmouth, Cardiff, and Dr. A. S. Horne of the Imperial College of Science and Technology, London.

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THE EFFECT OF SALICYLIC ALDEHYDE ON THE INFECTION OF WHEAT BY *PYTHIUM ARRHENOMANES* DRECHSLER, AND THE DESTRUCTION OF THE ALDEHYDE BY *ACTINOMYCES ERYTHROPOLIS* AND *PENICILLIUM* SP.[1]

By V. E. Graham[2] and L. Greenberg[3]

Abstract

Salicylic aldehyde, when added to soil at the rate of 50 p.p.m., seems to predispose wheat roots to attack by parasitic strains of *Pythium arrhenomanes*.

Actinomyces erythropolis and a species of *Penicillium* have been found in soil from the healthy area of a field partially infected with Browning root rot. These organisms caused the disappearance of salicylic aldehyde in an artificial medium.

It is suggested that lack of activity on the part of such organisms in certain areas of a field may lead to an accumulation of salicylic aldehyde or products acting in a similar manner, and that this may be a predisposing factor in the appearance of Browning root rot caused by *Pythium arrhenomanes*.

Introduction

During an investigation of host-conditioning factors that influence the severity of root rot of sugarcane by *Pythium arrhenomanes*, Rands and Dopp (3) found that the presence of salicylic aldehyde in the soil increased the susceptibility of the cane to fungus attack. Concentrations of salicylic aldehyde of 20 and 40 p.p.m. parts of soil had little influence on cane growth in the absence of the fungus, but when the fungus was present an apparent predisposition of the roots to attack became evident. A reduction in weight of plants was obtained that amounted to two to seven times the reduction found when only the fungus was present. The prevalence of *Pythium* root rot of sugarcane on the heavy, poorly drained soils of Louisiana was thought to be due, in part at least, to the presence of this and similarly behaving compounds. Improvement in drainage and general fertility in such areas was reported to increase markedly yields of cane.

Shorey (4) showed that salicylic aldehyde was present in some soils and attributed its presence to the addition of the aldehyde itself or to bodies yielding it in vegetative remains. Skinner (5) mentioned that aldehyde (not necessarily salicylic aldehyde) was more commonly found in acid soils, but that it was also obtained in certain cases from neutral and alkaline soils. He found that salicylic aldehyde was not harmful to the crops tested in culture solutions in amounts of 10 p.p.m., but that 25 p.p.m. was decidedly harmful. In soils, 50 p.p.m. was injurious to corn in both sand and clay soils, the effect being more marked in sand. Soils with strong oxidizing power (as determined by the oxidation of aloin) overcame the effect of the aldehyde, and the theory was advanced that the destruction of the added aldehyde was due to biological processes. In the soils tested, the effect of the added salicylic

aldehyde was overcome by the application of lime.   Phosphorus also reduced the damage in all cases and eliminated it entirely in some.   Field experiments verified these results, and indicated that the aldehyde persisted in some soils and disappeared in others and that this could be linked to the oxidizing power of the soil.   The soils in which the aldehyde was destroyed had strong biological activities, good oxidizing powers, and were fertile.   Salicylic aldehyde was found to occur more frequently in soils of low productivity.   No mention is made in any of these studies of disease having an influence on the results obtained.   In solution cultures, however, it was found that the addition of 10 p.p.m. of manganese completely overcame the adverse effect of the added aldehyde and that the roots were less stunted in the presence of lime.

Browning root rot of wheat in Saskatchewan is caused primarily by *Pythium arrhenomanes* (7).   There has been observed, in central and northern Saskatchewan, a tendency for the diseased patches of a field to occupy slightly depressed positions as compared to the uninfected portions.   The moisture content of the soil in these Browning areas is usually higher than that of corresponding disease-free areas of the same field.   This may be explained partly or wholly by the lowered demand on soil moisture due to the stunted growth.   On the other hand, it is not uncommon to find fields of rolling topography in which the wheat plants are healthy on the higher and lower areas and infected on areas of intermediate elevation.   This condition is not comparable to a heavy, poorly drained soil as mentioned by Rands and Dopp (3).   The exact relation of drainage to the incidence of Browning root rot is not clear.

Experimental

Salicylic Aldehyde as a Predisposing Factor to Pythium Damage

The results obtained by Rands and Dopp (3), Shorey (4), and Skinner (5), suggested the need for a study of the effect of salicylic aldehyde on the severity of attack of wheat roots by *Pythium arrhenomanes*.   To determine whether the aldehyde would predispose wheat to attack by the fungus the following experiment was devised. A weighed amount of soil (1500 gm.) was placed in each of eight pots.   These were sterilized in the autoclave at 20 lb. pressure for four hours and then in the hot air oven at 160° C. for two hours.   Duplicate pots were treated according to the following scheme:

    A. Controls—not treated.

    B. Salicylic aldehyde added to give a concentration of 50 p.p.m.

    C. Salicylic aldehyde, 50 p.p.m.   Inoculated with *Pythium arrhenomanes*, strain SH_2.

    D. Inoculated with *Pythium* only.

Two days after treatment, 20 disinfected wheat seeds were planted in each pot at a depth of one inch.   The fungus was grown on a mixture of sterile soil and cornmeal, and a blank of this medium was added to the other pots to eliminate any difference due to the cornmeal.   The resulting crops produced

in the greenhouse were photographed when three weeks old and are shown in Fig. 1. The apparent effect of salicylic aldehyde in intensifying the injury produced by the fungus is evident in the illustration.

Isolation of Organisms That Decompose Salicylic Aldehyde

Skinner (5) referred to the disappearance of salicylic aldehyde from the soil and mentioned that this was due to biological processes. No mention was made of specific organisms capable of producing this result. Two such organisms were isolated by the following simple procedure. A nutrient solution was prepared that contained the salts used by Thornton (6) in counting bacteria in soil, but with the agar and mannitol eliminated. This
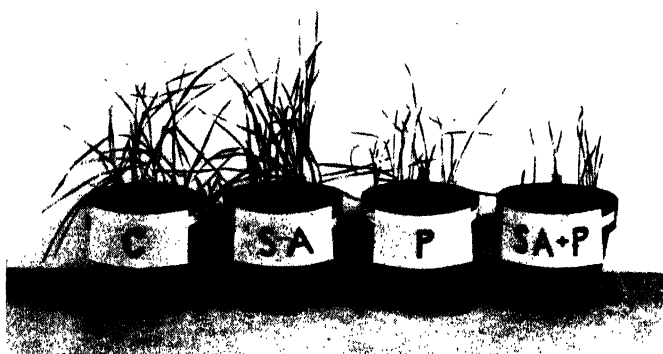
FIG. 1.   C - control, sterilized soil; SA - sterilized soil plus 50 p.p.m. salicylic aldehyde (Eastman Kodak Co.); P - sterilized soil plus inoculum of Pythium arrhenomanes; SA + P - sterilized soil plus 50 p.p.m. salicylic aldehyde plus inoculum of Pythium arrhenomanes.

was sterilized in 200-ml. quantities in 500-ml. flasks. Salicylic aldehyde was added after sterilization to give concentrations of 10 and 20 p.p.m., respectively. This medium was inoculated with a suspension of organisms obtained by shaking up 25 gm. of soil from the healthy area of a partially infected field, allowing the coarser particles to settle, and withdrawing 0.1 ml. of the relatively clear suspension. After three weeks' incubation at room temperature, the material in the flasks was plated on Thornton's agar for bacteria and on peptone-glucose-acid agar for moulds. No moulds were obtained from the flasks containing 20 p.p.m. of aldehyde and only one type appeared on the plates from the 10 p.p.m. flasks. Sixteen cultures of bacteria were isolated from the plates.

The individual ability of each of these crude cultures to destroy salicylic aldehyde when grown on a mineral-salt–salicylic-aldehyde mixture was then tested. After 4 days' incubation at room temperature the salicylic aldehyde had disappeared from 4 tubes; after 11 days' incubation it had disappeared

from 2 more, making a total of 6 positive aldehyde-destroying cultures out of the 16 tested. The aldehyde had not been destroyed by the mould culture in 4 days but was destroyed after 11 days. Through the courtesy of Dr. J. E. Machacek, of the Dominion Laboratory of Plant Pathology, Winnipeg, the mould culture has been tentatively identified as belonging to the *Penicillium janthinellum* series and is probably *P. Rivolli Zaleski*.

Microscopic examination of the bacterial cultures indicated that there was a Gram-positive branching organism present in all those that caused the disappearance of salicylic aldehyde. This organism apparently belonged to the *Actinomycetaceae* (Bergey (1)). Culture No. 7 was a pure culture of this organism, and it has been identified as *Actinomyces erythropolis* (Gray and Thornton) (2), (Bergey) (1). This organism was originally isolated by Gray and Thornton (2), who reported that it caused the breakdown of phenol and m-cresol. A comparison of Culture No. 7 with the organism of Gray and Thornton proved that they are identical.

Discussion

This study of the effect of salicylic aldehyde when added to soil and its relation to the attack of wheat roots by *Pythium arrhenomanes* was commenced too late in the season to allow any field work to be done on the subject. The fact, however, that salicylic aldehyde was found in many soils by Skinner (5), coupled with the observation that the addition of this aldehyde to soil seems to increase the susceptibility of the roots of both wheat and sugarcane to attack by *Pythium arrhenomanes*, is considered to be very suggestive. It remains to be seen whether salicylic aldehyde can be isolated in larger quantities from diseased areas under natural conditions, than from unaffected soil. The effect of the salicylic aldehyde seems to be on the plant rather than on the fungus, as shown by Rands and Dopp (3) and also by our experiments in which it was proved that the fungus could not grow on a medium containing 10 p.p.m. of salicylic aldehyde—an amount which was without effect on wheat.

Vanterpool (7) has shown that the addition of phosphorus fertilizer to soil eliminates serious damage to wheat from *Pythium arrhenomanes*. Skinner (5) showed that the deleterious effect of salicylic aldehyde in soil was lessened by the application of phosphorus and that 10 p.p.m. of manganese overcame it entirely in solution cultures. These results are very interesting, and it is suggested that there should be determinations made of the amount of manganese in healthy and diseased soil and in phosphorus fertilizers. It is quite possible that other substances, acting somewhat as catalysts, would have an effect similar to that of manganese. A study of the effect of some of these substances on the growth of *Actinomyces erythropolis* is also suggested.

This preliminary study suggests that when soil conditions are such as to inhibit the growth of organisms that are capable of breaking down salicylic aldehyde, this substance will accumulate in the soil and contribute to the severity of the attack on the roots by *Pythium arrhenomanes*. It is realized

that other substances may be capable of predisposing wheat to attack in a similar manner. In any case, micro-organisms have been found in soil that can destroy salicylic aldehyde, although the manner in which the aldehyde is destroyed is not known. It is apparent that qualitative relations between the flora of infected and uninfected areas may be of importance in determining the incidence of the disease. This hypothesis affords a reasonable explanation of the tendency for Browning disease to appear in isolated areas of an otherwise healthy field.

Acknowledgments

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OBSERVATIONS ON THE STUDY OF VARIETAL DIFFERENCES IN THE MALTING QUALITY OF BARLEY. PART IV[1]

By HENRY R. SALLANS[2] AND J. ANSEL ANDERSON[2]

Abstract

An investigation was undertaken to compare the relative magnitudes of the differential effects of environment (*i.e.*, stations) and of malting methods on the malting quality of barley varieties. Samples of Olli, O.A.C. 21, Hannchen, and Regal from four stations were malted by eight methods representing the combinations of germinating at 50° and 56° F., at 42 and 46% moisture, and for six and nine days.

Statistical analyses showed that, with respect to diastatic activity, wort nitrogen, and extract yield, the variations due to the differential effect of environment on varieties were greater than the variations due to the differential effect of malting methods on varieties. As a result of this study and earlier ones, it appears that the former effect is the limiting factor in studies of the comparative malting qualities of varieties. The latter effect is of less importance but must be kept in mind if errors in the interpretation of the results of routine tests are to be avoided. In general, it appears that if routine tests show that the mean values for any variety, when grown at 12 stations representing a reasonable range of environment, differ from the values for the standard variety by more than 1% in extract, or 10% in diastatic activity or wort nitrogen, a real difference exists between the varieties, which cannot be overcome by any reasonable change in malting conditions.

As a result of previous studies in this series (2, 3, 5), it has been suggested that the differential effect of malting methods on varieties may be an appreciable source of error in the interpretation of the results of routine tests in which all samples are malted by one method. It seems probable, however, that the limiting factor in studies of the comparative malting qualities of varieties is the differential effect of environment on them.

Theoretically it should be possible to make an adequate test of this hypothesis by means of one large investigation involving malting, under a wide range of conditions, samples of a large number of varieties grown at a number of points representing a wide range of environments. In practice, the difficulty of obtaining a suitable series of sufficiently large samples of barley, the limited capacity of available malting equipment, and the amount of time and number of analysts required, make it almost impossible to carry out one single investigation of adequate size. It has accordingly been necessary to resort to the use of several small investigations. Considered individually

these are not particularly convincing, but considered together, they provide a good deal of support for the hypothesis.

This paper presents the results of a fourth study in which samples of four barley varieties from four experimental stations were malted by eight methods. An attempt was made to determine whether circumstances might exist in which the differential effect of malting methods on varieties, rather than the differential effect of environment on varieties, would be the limiting factor in investigations of varietal differences in barley. For this reason an attempt was made to exaggerate the former effect. The malting treatments were selected to cover a very wide range of conditions: from a 6-day period of slow growth at 50° F., 42% moisture and with minimum aeration, to a 9-day period of rapid growth at 56° F., 46% moisture and with liberal aeration. All the malts grown under the first set of conditions were definitely under-modified, whereas all those grown under the last set of conditions were over-modified. The range of conditions was thus wider than that which would be selected for making commercial malts from these barleys, and the differential effect of malting methods on varieties was thus exaggerated.

Moreover, further exaggeration was obtained by using previous information on some 20 varieties grown in Canada to select four that promised to differ very widely in their reactions to changes in malting conditions, including amongst these one two-rowed variety, Hannchen. In our opinion most random selections of four varieties would tend to show less differential response than the four varieties used in the present study. This would apply particularly to sets composed entirely of six-rowed or entirely of two-rowed varieties. In general, except for certain experimental purposes, it seems unwise to attempt to compare the malting qualities of two- and six-rowed varieties by malting them by the same method, since, if this is satisfactory for one class, it will hardly do justice to the other. The results showed that the two-rowed variety, Hannchen, contributed more than its share to the interaction between malting treatments and varieties, particularly with respect to malting loss and wort nitrogen. Thus if Hannchen had been replaced by a six-rowed variety, it seems probable that the differential effect of treatments on varieties would have been reduced.

On the other hand, no attempt was made to exaggerate the differential effect of environment on varieties. So far as can be determined, selection of four varieties which differed widely in their reactions to changes in malting conditions, did not also result in selection of varieties which differed abnormally in their reactions to changes in environment. In addition, it appears that the four stations selected represent a fair sample of the range of environments over which varieties are commonly compared in Canada, and the differential effect of stations on varieties is of the order usually found in investigations of this type.

It is thus apparent that in the present investigation a definite attempt was made to disprove the hypothesis in which we are interested, namely, that the differential effect of environment on varieties, rather than the differential

effect of malting treatment on varieties, is the limiting factor in studies of the comparative malting qualities of different barley varieties. In spite of this attempt the results served to substantiate the hypothesis with respect to diastatic activity, wort nitrogen and extract.

The investigation was so designed that certain interrelations between the effects of germination time, temperature, and moisture could be investigated by statistical methods. Although the results thus obtained have little bearing on the hypothesis under investigation, they appeared to be worth reporting briefly.

Materials

The following four varieties were selected for study: O.A.C. 21, the six-rowed, rough-awned Manchurian type variety which is accepted as the standard of malting quality in Canada; Olli, a similar variety to O.A.C. 21 but of earlier maturing habit and with rather smaller kernels, which seems promising from the malting viewpoint; Regal, a six-rowed, smooth-awned variety which Canadian maltsters consider unsatisfactory; and Hannchen, a two-rowed, rough-awned variety of which considerable quantities are malted in the United States.

Samples of these four varieties, grown in adjacent plots, were obtained from four experimental stations, namely: Nappan, Nova Scotia; Kapuskasing, Ontario; Ottawa, Ontario; and Lacombe, Alberta. The thin kernels were removed from the samples by passing them over a ring grader with rings 5/64 in. apart. A Boerner sampler was used in preparing sub-samples.

Equipment and Methods

Malting Equipment

The malts were made in laboratory equipment at the National Research Laboratories, Ottawa. One steep-tank for 16 samples, two germinators, each for 8 samples, and one kiln for 8 samples, were available for the investigation. The germinators and kiln have already been described (1).

New steeping equipment was built just before the present investigation was started and will be described in detail in a future paper. It provides for automatic aeration of samples and automatic changing of steepwater. In addition it permits the steeping of individual samples to be started automatically at predetermined times (within ±5 min.). This feature, in conjunction with pilot experiments designed to determine the length of time required to bring a sample to the required moisture content, makes it readily possible to steep all samples to within ±0.5% of the required moisture content at the same time. The exact moisture content required can then be obtained by adding a few grams of water by sprinkling with an atomizer or by removing a few grams with blotting paper. For the present investigation, samples representing 250 gm. of barley dry matter were steeped to 42 or 46% moisture at 50° F., with a change of water and 1 hr. of aeration every 12 hr.

Malting Treatments

The eight malting treatments used consisted of the combinations of:—two germination times, 6 and 9 days; two germination temperatures, 50° and 56° F.; and two moisture levels, 42 and 46%.

In order to accentuate differences in growth rate, the samples that were steeped to 42% moisture were given less aeration and no watering. They were germinated in cylindrical galvanized iron containers, 6 in. long by 6 in. in diameter, containing eight $\frac{1}{8}$-in. holes. Aeration and evaporation were thus reduced to a minimum and the samples grew relatively slowly but continuously and without requiring water. The samples that were steeped to 46% moisture were germinated in 200-hole containers. Considerable aeration was thus provided, and as evaporation took place all malts were sprinkled with 20 gm. of water after 72 hr., and the 9-day malts were sprinkled a second time with 10 gm. of water after 144 hr.

All malts were dried in the same kiln in rotating wire-mesh cages under the following time-temperature schedule: 0 to 6 hr., temperature rising at constant rate from 90° to 125° F.; 6 to 12 hr., at 125° F.; 12 to 16 hr., temperature rising at constant rate to 140° F.; 16 to 21 hr., at 140° F.; 21 to 22 hr., temperature rising at constant rate to 170° F.; 22 to 26 hr., at 170° F.

Malting Plan

It seemed best to make in each batch the 32 malts representing eight treatments applied to four varieties from one station. This could be done as follows: by germinating four samples steeped to 42% moisture and four samples steeped to 46% moisture in one chamber at 50° F.; by germinating a corresponding set of eight samples in the second chamber at 56° F.; and by removing and kilning half of each sample after six days and the remaining halves after nine days. However, this procedure could not be followed, since it involved kilning 16 half-samples simultaneously whereas the kiln accommodated only eight half-samples. It was accordingly necessary to malt the half-batch grown at 56° F. five days later than the half-batch grown at 50° F. This schedule made it possible to remove the green malts in lots of eight and thus to dry all malts in one kiln.

Four batches, one for each station, were required to make one set of 128 malts, and four more batches were required to make the duplicate set. The first and second sets of four batches were malted in random order, and the samples were also arranged in random order within half-batches.

Analytical Methods

The moisture content and extract yield of the malts were determined by the Official Methods of the American Society of Brewing Chemists. Diastatic activity was determined by a ferricyanide modification of the official method (4). Wort nitrogen was determined by a Kjeldahl determination made on 25 ml. of wort after acidification and evaporation to a thin syrup, and is

reported as percentage of malt dry matter. Malting loss was calculated from data on the dry weights and moisture contents of the barley samples and the finished malts.

Single determinations of diastatic activity and malting loss were made on each of the duplicate malts. The precision of the malting methods was thus established by use of the two properties most sensitive to variations in malting conditions. Owing to press of other work the remaining portions of duplicate malts were mixed and single determinations of extract and wort nitrogen were made.

Results

Diastatic Activity

The results for diastatic activity are presented in Fig. 1 by means of a family of 25 histograms. These are arranged in tabular form, the columns showing the results for different malting treatments and the means over all treatments, and the rows showing the results for samples from different stations and the means over all stations.

Each individual histogram contains four columns which represent the four varieties: from left to right, Olli, O.A.C. 21, Hannchen, and Regal. The
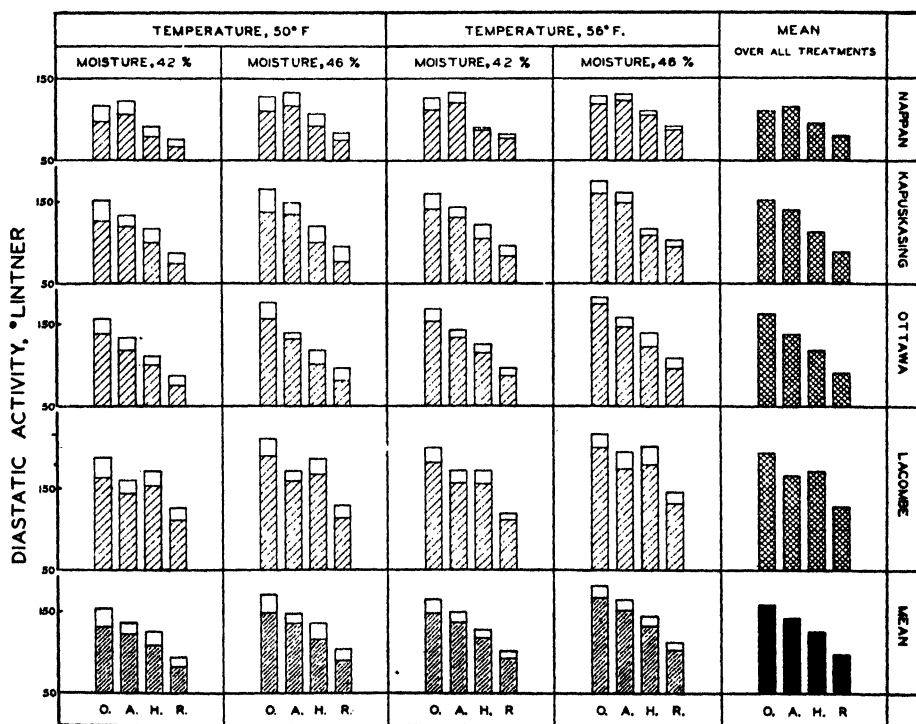
FIG. 1. *Histograms for diastatic activity illustrating the differential effects of stations and malting treatments on varieties. The four columns in each histogram represent four varieties: O = Olli, A = O.A.C. 21, H = Hannchen, and R = Regal. Tops of hatched portions represent six-day malts and tops of blank portions represent nine-day malts.*

hatched portion of each column represents the results for six-day malts and the white portion represents the additional effect of growing the malts for three more days. It should be noted that all histograms are coded, since the base lines represent 50° rather than 0° Lintner.

It is probably best to consider first the histogram in the lower right-hand corner, which presents the mean values for each variety over all stations and malting treatments. The varieties fall in the following order: Olli, 158° Lintner; O.A.C. 21, 142° L.; Hannchen, 125° L.; and Regal, 97° L.

By considering the five histograms in the last column we can now determine: (i) whether differences exist between the average values for each station; and (ii) whether the varieties fall in the same order at each station. Nappan samples gave the lowest average value, 105° L.; samples from Kapuskasing and Ottawa gave medium values of about the same order, 123° and 128° L.; and samples from Lacombe gave the highest average value, 165° L. More detailed comparisons of the five histograms will show that at Kapuskasing and Ottawa the varieties fell in the order shown by the means (black histogram); at Nappan the main discrepancy from this order is that Olli gives a lower value than O.A.C. 21, and at Lacombe the main discrepancy is that Hannchen gives a higher value than O.A.C. 21. It is thus apparent that the differential effect of stations (*i.e.*, environment) on varieties is very considerable.

The bottom row of histograms illustrates: (i) the mean differences between malting treatments; and (ii) the differential effect of treatments on varieties. It is apparent that diastatic activity was increased by increasing the germination time, by increasing the moisture content, and by raising the temperature. Since the configurations of all histograms in the bottom row are very similar, it is obvious that there was comparatively little interaction between treatments and varieties. It is apparent, however, that there was some interaction. For instance, in general, a change in treatment that tended to increase diastatic activity had a greater effect on Olli than on Regal.

The remaining 16 histograms (first four rows and first four columns) present the results for each treatment at each station. Comparison of these within rows shows the interaction between varieties and treatments within stations, and comparisons within columns show the interaction between varieties and stations within treatments. Since the histograms are very similar in configuration within rows, whereas considerable differences exist between the configurations of histograms in different rows, there can be no doubt that with respect to diastatic activity the differential effect of stations on varieties was considerably greater than the differential effect of malting treatments on varieties.

Wort Nitrogen

The family of histograms for total wort nitrogen as percentage of malt dry matter is shown in Fig. 2. These are arranged in exactly the same order as

those in Fig. 1. They are also coded: the base lines represent 0.65% rather than 0%.

The histogram with black columns shows that with respect to mean values over all stations and treatments, the varieties fall in the following order: Olli, 1.00%; O.A.C. 21, 0.89%; Hannchen, 0.87%; and Regal, 0.84%. The mean values for stations over all varieties and treatments are: Nappan, 0.80%; Kapuskasing, 0.86%; Ottawa, 1.00%; and Lacombe, 0.94%.

FIG. 2. *Histograms for wort nitrogen illustrating the differential effects of stations and malting treatments on varieties. For additional explanation see title of Fig. 1.*

The last column of histograms shows that the varieties fell in different orders at different stations. The configuration of the histogram for Kapuskasing is almost identical with that for the means. At Nappan, O.A.C. 21 gives as high a value as Olli; at Ottawa, Hannchen gives considerably higher values than O.A.C. 21 and Regal; and at Lacombe, Regal gives a slightly higher, rather than a lower, value than Hannchen. Thus with respect to wort nitrogen, it is apparent that environment has a considerable differential effect on varieties.

The differential effect of malting treatments on varieties is illustrated by the last row of histograms. If only the six-day malts are considered (hatched portions of columns only) it is apparent that the histograms have much the

same configurations. A fair degree of similarity also exists between the histograms for nine-day malts (tops of columns), although it will be observed that a change in moisture content has a considerable effect on configuration. The configurations of the histograms for the six- and nine-day malts differ considerably. It is thus apparent that the interaction between varieties and germination time is fairly large, and is greater than the interaction between varieties and moisture content, and that this again is greater than the interaction between varieties and germination temperature.

Further inspection of the histograms in the bottom row will show that the two-rowed variety Hannchen contributed most to the differential effect of treatments on varieties. It showed a far greater response to the increase in germination time than any of the other varieties. O.A.C. 21 also contributes, since it tends to respond least to changes in treatment.

By considering the remaining 16 histograms by rows, the interaction between varieties and treatments within stations can be elucidated. It will be observed that at Nappan, O.A.C. 21 samples grown at 56° F. gave a lower percentage of wort nitrogen after nine than after six days of germination. A similar tendency for the percentage nitrogen to remain constant or decrease slightly, with an excessive increase in germination time, was observed in a study of a low nitrogen sample of O.A.C. 21, reported in Part II of these studies (2). It will also be observed that at Ottawa, Hannchen showed a far greater increase in wort nitrogen with increasing germination time than it did at the other three stations.

A general comparison of the differences in the configurations of histograms within and between rows, special attention being paid to the last row and the last column, leaves little doubt that the differences between rows are greater and thus that the differential effect of stations on varieties is appreciably greater than the differential effect of malting treatments on varieties.

Extract

The coded histograms (base line, 67.5%) for extract are presented in Fig. 3.

The mean values for varieties over all stations and treatments are: Olli, 77.6; O.A.C. 21, 75.6; Hannchen, 78.0; and Regal, 72.9.%. The mean values for stations, over all varieties and treatments, are: Nappan, 77.9; Kapuskasing, 76.3; Ottawa, 75.8; and Lacombe, 74.3%.
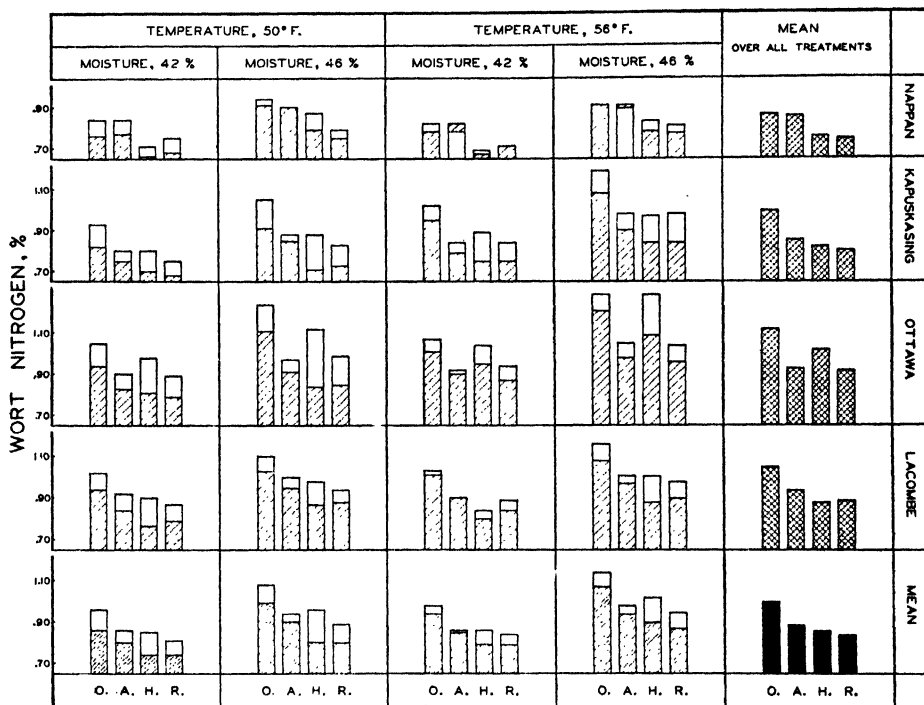
The last column of histograms again shows that the differential effect of stations on varieties is quite large. Hannchen is about equal to Olli at Nappan, appreciably higher at Kapuskasing and Ottawa, and appreciably lower at Lacombe. These two varieties yield considerably more extract than O.A.C. 21 except at Lacombe, where Hannchen is only slightly above O.A.C.21. Regal yields the lowest value at all stations but is not much lower than O.A.C. 21 at Kapuskasing.

The differences in the effects of treatments are particularly obvious in this family of histograms. At the higher temperature and moisture level (fourth

column), the nine-day malts yielded less extract than the six-day malts, whereas with other treatments the reverse holds true. It is thus apparent that the malts made at the higher temperature and moisture level were very much overgrown at nine days, with the result that the extract yield was decreased by excessive malting loss. Results of this type were also obtained in the investigation reported in Part II of this series (5) and are there illustrated by curves in which extract yield is plotted against germination time.
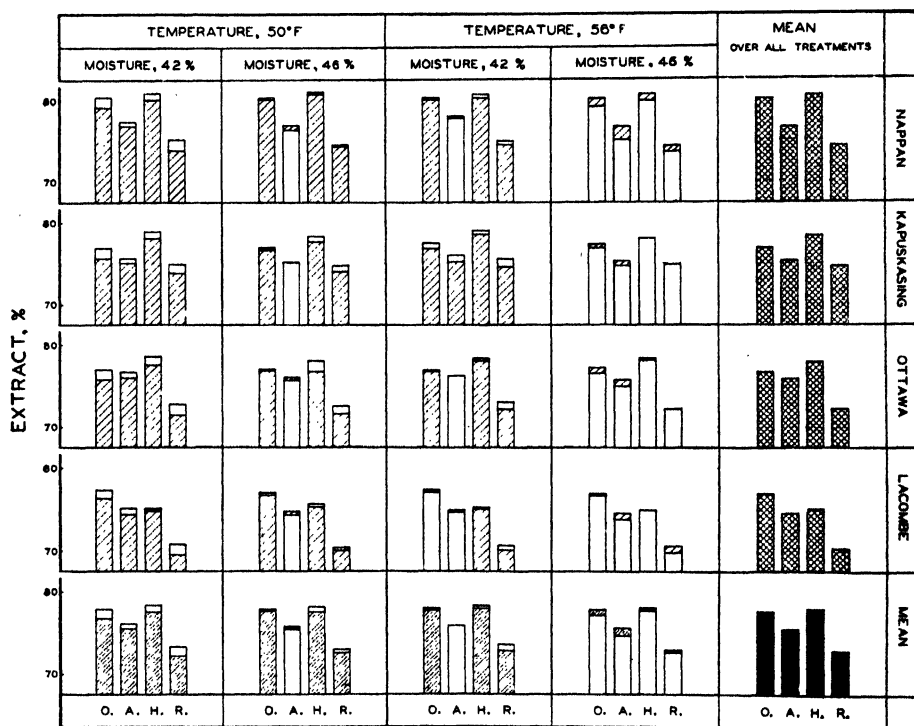
FIG. 3. *Histograms for extract illustrating the differential effects of stations and malting treatments. For additional explanation see title of Fig. 1.*

Inspection of the individual rows of histograms will show that there is an appreciable interaction between varieties and malting treatments. This is particularly noticeable with respect to the differences between six- and nine-day malts made at 50° F. and 46% moisture. At three out of four stations, O.A.C. 21 yielded less extract at nine than at six days, whereas the other varieties, particularly Hannchen and Regal, yielded more extract at nine than at six days, at all stations. The results for 56° F. and 46% moisture are similar, since the decrease in extract from six to nine days is greater for O.A.C. 21 than for any of the other varieties.

In general it is quite apparent that the differences between the configurations of histograms within rows are much less than the differences between the

configurations of histograms in different rows.   It follows that with respect
to extract, the differential effect of malting treatments on varieties was much
lower than the differential effect of stations on varieties.

Malting Loss

The coded histograms (base line at 5%) for malting loss are presented in
Fig. 4.

The mean values for varieties, over all stations and malting treatments,
are: Olli, 10.8; O.A.C. 21, 10.7; Hannchen, 11.1; and Regal, 10.1%.   The
mean values for stations, over all varieties and treatments, are: Nappan, 10.1;
Kapuskasing, 10.4; Ottawa, 10.7; and Lacombe, 11.6%.
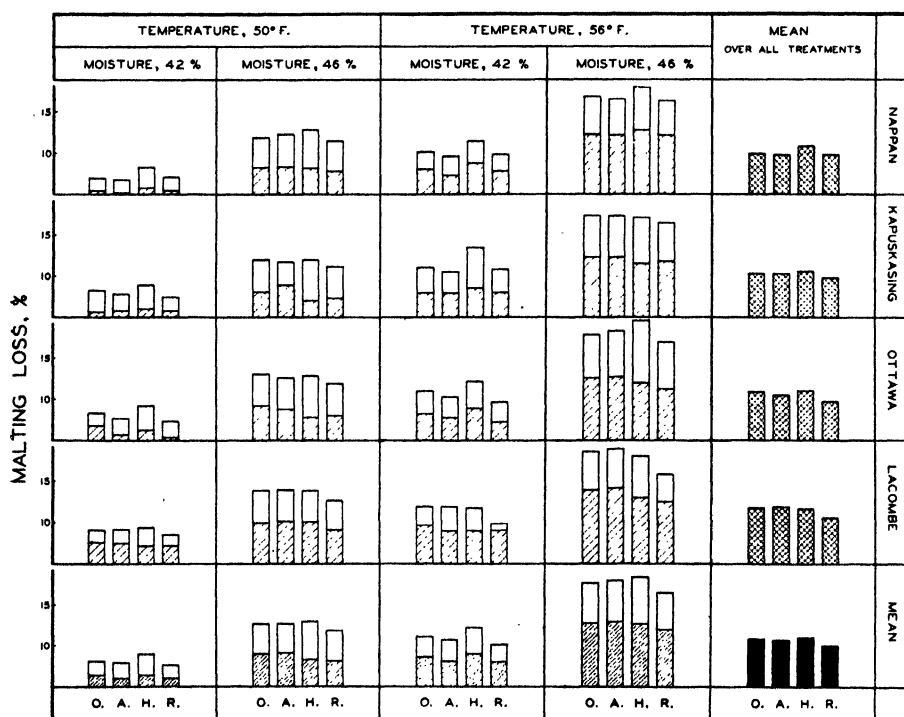
FIG. 4.   *Histograms for malting loss illustrating the differential effects of stations and malt-
ing treatments on varieties.   For additional explanation see title of Fig. 1.*

Inspection of the last column of histograms will show that there was some
interaction between stations and varieties.   The relations between Hannchen
and Regal remain about the same at all stations.   On the other hand, by com-
parison with Hannchen, Olli and O.A.C. 21 give much lower values for Nappan
and slightly higher values for Lacombe.

The bottom row of histograms again illustrates the differential effect of
treatments on varieties.   Considering the six-day malts only, it is apparent

that at the lower moisture levels (first and third columns of histograms) O.A.C. 21 gives lower values than Olli or Hannchen, whereas at higher moisture contents (second and fourth columns) O.A.C. 21 gives higher values. Again considering the six- and nine-day malts, it is apparent that the additional three days' growth resulted in a greater increase in the malting loss for Hannchen than in the malting losses for the other three varieties.

In general, the differences between the configurations of histograms within rows are of about the same order as the differences between rows. It accordingly appears that with respect to malting loss, the differential effect of malting treatments on varieties is of about the same magnitude as the differential effect of stations on varieties.

Interactions Between Germination Time, Temperature, and Moisture Level

The investigation also brought to light certain interactions between germination time, temperature, and moisture level. These effects cannot be readily elucidated by study of the histograms in Figs. 1 to 4 and data illustrating them are therefore given in Table I. The data presented are mean values for all samples.

TABLE I

DATA FOR DIASTATIC ACTIVITY, WORT NITROGEN, EXTRACT, AND MALTING LOSS, ILLUSTRATING INTERACTIONS BETWEEN GERMINATION TIME, TEMPERATURE, AND MOISTURE LEVEL

Property	Time × temperature			Time × moisture			Moisture × temperature		
	Time	Temperature		Time	Moisture		Moisture	Temperature	
		50° F.	56° F.		42%	46%		50° F.	56° F.
Diastatic activity	9 days	133	143	9 days	131	145	46%	130	144
	6 days	116	130	6 days	117	130	42%	119	129
	Difference	19	13	Difference	14	15	Difference	11	15
Wort nitrogen	9 days	.893	.956	9 days	.909	.996	46%	.866	.984
	6 days	.829	.919	6 days	.813	.880	42%	.827	.921
	Difference	.064	.037	Difference	.096	.116	Difference	.039	.063
Extract	9 days	76.3	76.0	9 days	76.5	75.9	46%	76.0	75.9
	6 days	75.7	76.1	6 days	75.8	76.0	42%	76.0	76.3
	Difference	0.6	−0.1	Difference	0 7	−0.1	Difference	0	−0.4
Malting loss	9 days	10.4	14.4	9 days	9.6	15.1	46%	10.6	15.1
	6 days	7.4	10.6	6 days	7.3	10.6	44%	7.2	9.8
	Difference	3.0	3.8	Difference	2.3	4.5	Difference	3.4	5.3

The interaction between time and temperature was quite marked with respect to each of the four properties studied. A three-day increase in germination time has a greater effect at 50° than at 56° F. on diastatic activity, wort nitrogen and extract, but the effect on malting loss is less at 50° than at 56° F.

The data also show that interactions existed between time and moisture, and moisture and temperature.

The data for extract are particularly interesting. It will be observed that at the lower temperature and at the lower moisture level, extract increased with an increase in germination time from six to nine days, whereas at the higher temperature and moisture level, extract decreased with increasing germination time. Similarly, a decrease in extract also took place at 56° F. when the moisture content was raised from 42 to 46%, whereas at 50° F. the change in moisture had no effect on extract yield.

The existence of these interaction effects emphasizes the difficulty of making accurate statements about the general effect on malting quality of a change in malting conditions. The effect of a change in any one malting factor such as germination time will depend upon the levels at which other factors such as temperature and moisture are maintained. Moreover, it will also depend upon the variety and total nitrogen content of the barley under investigation.

Statistical Analyses

As a first step in the statistical analyses, the variance of the data for each determination was analysed into portions due to: (i) average differences between varieties; (ii) average differences between stations; (iii) average differences between malting treatments; (iv) differences in the relative average performance of varieties at different stations; (v) differences in the relative average performance of varieties under different treatments; (vi) differences in the relative average performance of stations under different treatments; (vii) the second order interaction between varieties, stations and treatments; and (viii) differences between duplicate malts (for diastatic activity and malting loss only). The results of these analyses are reported in Table II.

TABLE II

ANALYSES OF VARIANCE FOR DIASTATIC ACTIVITY, WORT NITROGEN, EXTRACT, AND MALTING LOSS

No.	Variance due to	Degrees of freedom	Mean squares			
			Diastatic activity	Wort nitrogen	Extract	Malting loss
i	Varieties	3	42,986**	.1697**	175.18**	12.63**
ii	Stations	3	40,843**	.2275**	70.84**	26.20**
iii	Treatments	7	4,947††	.0924††	1.87††	416.567††
iv	Varieties × stations	9	1,347‡‡	.0123‡‡	8.41‡‡	1.33‡
v	Varieties × treatments	21	76**	.0022**	.26**	1.20**
vi	Stations × treatments	21	58**	.0049**	.18**	1.18**
vii	Varieties × stations × treatments	63	18	.0007	.05	.28
viii	Duplicate malts	128	6	–	–	.38

**Significantly greater than (iv); ††Significantly greater than the larger of (v) and (vi); ‡Significantly greater than the larger of (vii) and (viii).

NOTE: In this and the following table, double signs denote that the 1% level, and single signs that the 5% level of significance is attained.

The statistics show that, with respect to each determination, the mean squares for varieties and stations are significantly greater than the mean square for the interaction between these two factors; that the mean square for malting treatments is significantly greater than the mean squares for the interactions between this factor and stations or varieties; and that the mean squares for the interactions between each pair of the three main factors are significantly greater than the mean square for the second order interaction between the three factors, which forms an estimate of the error of the investigation.

As the investigation was undertaken with the main object of comparing the interaction between varieties and stations with the interaction between varieties and treatments, particular attention should be paid to the corresponding mean squares. The ratios of the mean squares for varieties $\times$ stations to the corresponding mean squares for varieties $\times$ treatments are as follows: for diastatic activity, 17.7; for wort nitrogen, 5.6; for extract, 32.6; and for malting loss, 1.1. It is thus apparent that, except with respect to malting loss, the differential effect of stations on varieties is a great deal larger than the differential effect of malting treatments on varieties.

As a further step in the analyses, the variance due to the interaction between varieties and malting treatments was analysed into portions due to: (i) differences in the relative performance of the varieties with different germination times; (ii) differences in the relative performance of the varieties at different moisture levels; (iii) differences in the relative performance of varieties at different temperatures; and (iv) a remainder, representing the second and third order interactions, which forms an appropriate estimate of error. The results of these analyses are given in Table III.

TABLE III

ANALYSES OF VARIANCE DUE TO INTERACTION BETWEEN VARIETIES AND MALTING TREATMENTS

No.	Variance due to	Degrees of freedom	Mean squares			
			Diastatic activity	Wort nitrogen	Extract	Malting loss
i	Varieties × times	3	170‡‡	.00843‡‡	.587‡‡	3.77‡‡
ii	Varieties × moisture	3	179**	.00387**	.464*	3.30**
iii	Varieties × temperatures	3	104*	.00120	.361*	1.10**
iv	Remainder	12	19	.00046	.097	.06

* and ‡‡ significantly greater than remainder mean square.

Of the three interactions studied, that between varieties and temperatures is lowest for all determinations. For wort nitrogen the interaction between varieties and times is highest, but for the other three determinations the interactions between varieties and times and between varieties and moisture levels are of much the same magnitude.

Discussion

The results of the investigation are quite clear-cut. The varieties did not fall in exactly the same order, with respect to any determination, when they were grown at different stations. It is thus clear that environment had a marked differential effect on varieties. Moreover, it is also apparent that when the malting treatments were changed, the relative positions of the varieties, with respect to each determination, were also appreciably changed. However, except with respect to malting loss, this differential effect of malting treatments on varieties was by no means as great as the differential effect of environment on varieties. The latter was thus the limiting factor in the study of the varieties, but in spite of it, significant differences were demonstrated between varietal means for each determination.

These conclusions apply specifically to the particular varieties, stations, and malting methods studied in the present investigation. Because of the small numbers of samples and methods, and because these do not represent *random* selections from larger homogeneous populations, there is some danger in arguing from the particular case reported in this paper to a more general one. It will be borne in mind, however, that by exaggerating the differential effect of malting treatment on varieties, but not the differential effect of environment on varieties, a deliberate attempt was made to disprove the hypothesis that the latter is greater than the former. Considerable weight must therefore be attached to the fact that in spite of this attempt, the results for diastatic activity, wort nitrogen, and extract provide strong support for the hypothesis.

With respect to malting loss the two differential effects were of the same order. This serves to emphasize the necessity for caution in interpreting data on this property. The matter has always been a difficult one. Differences between varieties are generally small by comparison with the precision with which they can be determined, though significant differences between certain pairs of varieties can frequently be demonstrated in spite of this limitation. It seems difficult to estimate the importance and significance of varietal differences in malting loss as determined under standardized conditions, and the results of the present investigation merely serve to increase the difficulties and emphasize the need for further research.

In Canada, the routine laboratory malting test is used for comparing newly developed or newly introduced varieties with the standard variety, O.A.C. 21. The test varieties and the standard are grown in small plots at a number of stations. The barleys are analysed, malted under standard conditions, and the malts are also analysed. Since the barley varieties do not fall in exactly the same relative positions at all stations, with respect to any barley or malt property (*i.e.*, since there is a differential effect of environment on varieties), the mean difference between a test variety and the standard must attain a certain magnitude before it can be considered significant. It has been our experience that fairly reliable estimates of differences in malting quality can be obtained by growing the varieties at twelve widely separated experimental

stations.   Under these conditions, mean differences of about 1% in extract, 10% in diastatic activity, and 10% in wort nitrogen, generally prove to be significant.   In other words, if differences of this order are found we should expect more reliable results, obtained by testing a far greater number of samples, to show conclusively that real differences exist between the varieties.

The conditions used in the routine test simulate those used in commercial plants in Canada and are thus adapted for malting average samples of the standard variety, O.A.C. 21.   The question that now arises is whether a test variety might not show up to better advantage if the malting conditions were changed.   As a result of this and earlier investigations (2, 3, 5), it appears that the spreads between certain pairs of varieties can be affected by changing the malting conditions.   This is particularly true of varieties that differ widely in malting characteristics, but if wide differences are shown to exist between any pair of varieties under one set of malting conditions, it seems probable that these will persist under all sets of conditions within a reasonable range.   In general, we believe that if the routine test shows that, on the average, a variety differs from the standard by more than 1% in extract, or 10% in diastatic activity or wort nitrogen, it will be found that a real difference exists between the varieties, which cannot be overcome by any reasonable change in malting conditions.   The routine test should therefore serve for the elimination of varieties that are definitely inferior to the standard with respect to one or more important malt qualities.   On the other hand, all varieties that the routine test shows to be reasonably promising will require further and more extensive study before it is possible to decide whether they are equal in malting quality to O.A.C. 21.

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THE ACCURACY OF THE PLATING METHOD FOR ESTIMATING THE NUMBERS OF SOIL BACTERIA, ACTINOMYCES, AND FUNGI IN THE DILUTION PLATED[1]

BY NORMAN JAMES[2] AND MARJORIE L. SUTHERLAND[3]

Abstract

During the crop seasons of 1936, 1937, and 1938, 1465 samples of field soil, held in the laboratory one day after crushing, were plated by the recognized technique in four replicates of one dilution for counts of fungi, and of a higher dilution for counts of bacteria and of actinomyces. A χ^2 value was calculated for each set of counts. These values for each group of micro-organisms were distributed into classes, and the number in each class was compared with the theoretical for the Poisson series. The data for each year indicate that the fungal counts conform to expectancy on the basis of random sampling, and show that the method provides a reasonably accurate estimate of the population in the dilution plated capable of developing under the conditions of growth. Too many sets of counts of bacteria in each year yield high χ^2 values. The counts of actinomyces conform to expectancy.

In an attempt to determine the cause of this abnormality for sets of counts of bacteria, samples were plated on the afternoon of the day they were taken from the field. Three hundred and four samples plated in six replicates of one dilution, and another 100 samples plated in four replicates, yield χ^2 values whose distributions conform to expectancy. Accordingly, the plate method provides a satisfactory estimate of the bacterial population of soil in the dilution plated if the procedure is carried out within six hours after sampling. Data on 88 samples plated on the day of sampling, on 88 samples held one day, on 88 samples held two to five days, and on 88 samples held eight to thirteen days show that the discrepancy between the actual and theoretical distributions of χ^2 values becomes progressively greater at each successive period of holding the samples. Further, the data indicate that the area sampled, the season, the medium used and the technique of plating bear no relation to the abnormal variation in counts of bacteria on replicate plates.

A record was kept of the presence of abnormal types of bacterial colonies and various genera of fungi on all plates from 468 samples plated one day after sampling and crushing during 1938. The data show that sets having pin-point colonies or spreading colonies of the Mucorales on one or more plates usually have high χ^2 values. Counts on such plates should be excluded from the estimate of the mean number of bacteria in the sample. Likewise, the number of actinomyces colonies on each plate from these samples was recorded. The χ^2 values for these counts were found to conform to expectancy, indicating that the factor or factors associated with a large number of high χ^2 values for counts of bacteria does not affect the count of actinomyces in the same way.

Percentage moisture and P values corresponding to χ^2 values for the counts of bacteria obtained each year were correlated. The data yield coefficients that are not significant in each case.

Introduction

The problem of estimating the numbers of micro-organisms in soil has engaged the attention of soil microbiologists since the beginning of the science and, after a half century marked by methods that are far from adequate, still has a fascinating appeal. The soil is known to be teeming with micro-

scopic life. There must be some way of estimating with reasonable accuracy the number of the various forms that exist in a given sample. The plate method, because of its wide use in early studies and because of its association with counts in other substances, particularly dairy products, still offers promise of providing results that have practical application. It is not without its limitations, which are recognized and cannot be considered lightly. The statement by Conn (5) that "It has in fact long been realized, even by those using these methods, that such counts have little significance; and they have come to be used less and less as bacteriologists have learned more about bacteria in the soil" represents an opinion that scarcely can be accepted as final. This investigator, supported in his opinion by Winogradsky (36, 37), favours the direct microscopic technique, which to date has not been given general approval and has not provided evidence of superiority (25). Thornton and Gray's (25) belief, in reference to the plating method, that "such counts have undoubted value in comparing two or more samples" appears to be shared by many who have continued to use the plating method, even since the development of the direct method. Among the foremost of these may be listed the following: Bisby *et al.* (1), Brierley *et al.* (2), Brown and Benton (3), Cobb (4), Corbet (6), Eggleton (7), Erdman (8), Gray *et al.* (14, 15), Harmsen and Verweel (16), Jensen (17), Lochhead and Thexton (18), Newton (19), Stevens (20), Taylor (22), Taylor and Lochhead (23), Timonin (24), Vandecaveye *et al.* (26–28), Waksman and Purvis (32), Williams (33), Wilson and Lyon (34), and Wilson and Kuhlmann (35).

Historical

The mathematical analysis of data obtained by the plating technique has engaged the attention of many investigators. Fisher *et al.* (9) developed a statistical control method for checking short series of parallel plates. They showed that, under ideal conditions of plating, the counts of bacterial colonies on parallel plates vary in the same manner as samples from the Poisson series; and that, when these conditions are fulfilled, the mean count of replicates is a direct measure of the density of the population. For a large number of sets of parallel plates, agreement with the theoretical distribution may be tested by

$$\chi^2 = \frac{S(x - \bar{x})^2}{\bar{x}}$$

where x is any number of colonies counted on a plate and $\bar{x}$ is the mean count of the replicates.

Waksman (29) found a probable error of 1.8% in counts of total bacteria, 2.7% in counts of actinomyces, and 5.8% in counts of fungi, when considering 50 plates from each plot for each group of micro-organisms. He presented, in addition, a table giving a weighted value based upon the number of samples from one source and the number of plates from the sample. To obtain a weight of 3.00, or the minimum for a very good rating, would require ten plates from each of three samples or two plates from each of ten samples.

Wilson and Kuhlmann (35) applied the χ^2 method to counts of rhizobia on Fred and Waksman's medium 79 (12) obtained by a standardized technique, and found that in data based on sets of three replicate plates the large values of χ^2 arose from counts in which one plate showed undue variation from the other two. In a second experiment in which five replicates were used, either the plate that showed marked deviation from the others, or the third plate when there was close agreement in all, was eliminated. In this case, the variations noted in the remaining plates could have arisen by chance alone.

Gray and associate (14, 15) prepared five replicates from each sample, but in some cases the count could be calculated from a smaller number of plates only. They show the number of plates used and the reliability of the results in relation to the χ^2 index of dispersion.

Jensen (17) applied statistical tests to counts of bacteria, actinomyces, and fungi in fifty samples of soil. He used formulae designed to test the variation when the number of replicate plates is not constant. The method does not verify the theoretical distribution with any exactitude but does test whether the general level of variability conforms with expectation (10). This investigator found the variation between parallel plates of bacteria to be very close to the expected, while that for replications of actinomyces showed a tendency to subnormality. He suggests that, since in the case of the actinomyces the difference is not very great, there is justification in considering the majority of the actinomyces counts as reliable. The test applied to the counts of fungi gave a difference within the permissible limits and he concludes that the technique must be considered as giving a reliable index of the numbers of mycelial fragments and fungal spores capable of developing on the medium used.

Harmsen and Verweel (16) applied Fisher's control test to a large number of series of replicate counts and presented histograms which indicate that numbers of bacteria found on parallel plates from field samples have an abnormal variance. This is proved true also on data published by Waksman and submitted to the test by these investigators. In this work, and in that of Waksman also, ten replicate plates from each sample were used. However, they found a very satisfactory distribution of χ^2 values on the data from actinomyces.

In a recent paper by Sutherland and James (21), the literature dealing with the problem of the application of the χ^2 test to bacterial counts by the plate method was reviewed. In this report it is shown that the technique of making dilutions and preparing plates produces a mean of four counts that is accurate as an estimate of the population in the dilution sampled when certain pure cultures are used. This finding is of interest from the standpoint of eliminating technique as the cause of the discrepancies in the χ^2 distributions referred to in previous reports.

Experimental

Scope of the Problem

The procedure for estimating the number of micro-organisms in the soil of a given field involves so many steps that there is probability of serious error. The work reported hereafter is an attempt to determine the accuracy of each step in the laboratory by the application of accepted mathematical tests to the data obtained and, where necessary, to provide a technique that is satisfactory for the purpose intended. Obviously, this necessitates starting at a point farthest from the field sample and progressing one step at a time. Accordingly, this presentation deals only with the accuracy of the mean count of four or six replicates from one dilution as an estimate of the population in the dilution plated.

A second paper will deal with the accuracy of the count from one dilution as an estimate of the population in the aliquot sample used in making the dilution, and the accuracy of the count from one aliquot sample as an estimate of the population in the sample brought to the laboratory.

I. The Accuracy of the Mean Count of Four Replicate Plates
from One Dilution: Bacteria

Method of Procedure

The 1465 samples used in this study were taken from two permanent series of plots in the experimental field of the Dominion Rust Research Laboratory at the University of Manitoba, in a long-term investigation of the microflora of grain-producing soils in relation to the cereal root-rot problem. The soil is a dark brown, heavy clay that becomes compact and sticky when wet. At many samplings the soil had a moisture content of over 40% and did not break up readily. Each laboratory sample was a composite of six cores, 6 by 1¼ in., taken at random from a hundredth-acre plot. It was crushed by careful hand manipulation and mixed thoroughly. A moisture test was run, and sufficient soil to give 25 gm. on a dry basis was added to 240 ml. of sterile water in a pint jar. This dilution was shaken for five minutes on a mechanical rocker shaker and given a vigorous shaking by hand immediately before a transfer was made. Higher dilutions were made in six-ounce, screw-topped medicine bottles and shaken by hand 25 times. The 1 : 200,000 dilution was used for plating in 1936. This was changed to 1 : 800,000 in 1937 and 1 : 500,000 in 1938. The higher dilutions seemed advisable from the standpoint of ease in counting. The number of colonies per plate ranged from 40 to 200, except in occasional samples where pin-point colonies raised the count. A one-millilitre sterile pipette was rinsed once in the dilution to be plated and one ml. was delivered to each of four plates.

Fred and Waksman's (12) sodium albuminate agar was used for bacteria and actinomyces. This medium has been used by Harmsen and Verweel (16) in a similar study, and by others. Incubation was at 25 to 28° C. for eight days. The higher temperature appeared necessary because of the impracticability

of maintaining a lower temperature during the excessive heat of midsummer. A mounted lens of four-inch diameter and two to three diameters magnification was used to check all plates before recording the counts.

Mean and χ^2 (Chi square) values were calculated for each set of counts on four replicates. The χ^2 values were distributed into classes on the basis of class boundaries for three degrees of freedom. Finally, the goodness-of-fit test (13) was used to ascertain the agreement between the actual and expected or theoretical distributions.

RESULTS

The goodness-of-fit test applied to the distribution of χ^2 values for counts of bacteria on 504 samples investigated in 1936 gives a final χ^2 value of 1336.08, with a P value decidedly below the 1% point. This finding was confirmed on 493 samples studied in 1937, and on 468 samples in 1938. The data for 1938 are presented graphically in Histogram 1.
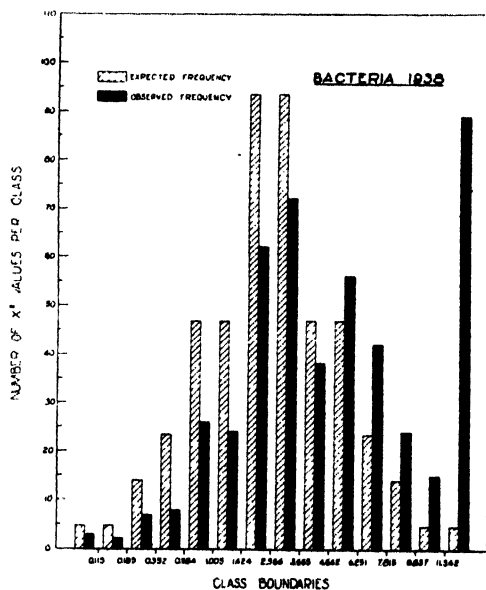
HISTOGRAM 1. χ^2 *distribution on 468 samples for counts of bacteria, 1938.*

These results show that the distributions of χ^2 values vary widely from expectancy. They point clearly to some factor or factors responsible for the exceedingly small P values in the data presented. A large series of sets of parallel plates should yield χ^2 values that are distributed according to the Poisson series, if the technique of dilution affords a perfectly random distribution of organisms, and if these can develop on the plate without mutual interference (10). A P value between 0.9 and 0.1 may be accepted as indicating that the variation among counts on replicate plates from one dilution may be attributed to chance.

II. Factors Contributing to the Abnormal Variation Among
Counts of Bacteria on Replicate Plates from One Dilution

(a) Time Sample Held in the Laboratory before Plating

The data obtained in the preceding section were obtained on samples held in the laboratory one day after they were broken up. This was carried on as a routine procedure to allow time for moisture determinations in order to obtain counts directly on the basis of the amount calculated to make 25 gm. of dry soil. Late in the summer of 1938 this procedure proved to be faulty from the standpoint of abnormal findings in the tests of variance to be considered in a later paper. As a consequence, data on 88 samples plated on the afternoon of the day of sampling were available for χ^2 distribution studies. The failure to find the usual abnormal distribution of χ^2 values prompted the continuance of the study of fresh samples as they affect the accuracy of the mean of four or six replicates from one dilution in conjunction with the tests of variance studies referred to above. Unfortunately, the record of how long others have held samples before plating is not made clear. The work of Harmsen and Verweel (16) gives no indication of their procedure in this respect. Likewise, the data furnished by Waksman (31) and analysed by these investigators, give no definite information on this point. Waksman (30) states that the samples were "plated as soon as possible", which allows for varied interpretations.

Results

The distribution of χ^2 values on 304 samples, each plated in six replicates from one dilution, is shown in Histogram 2. The goodness-of-fit test applied
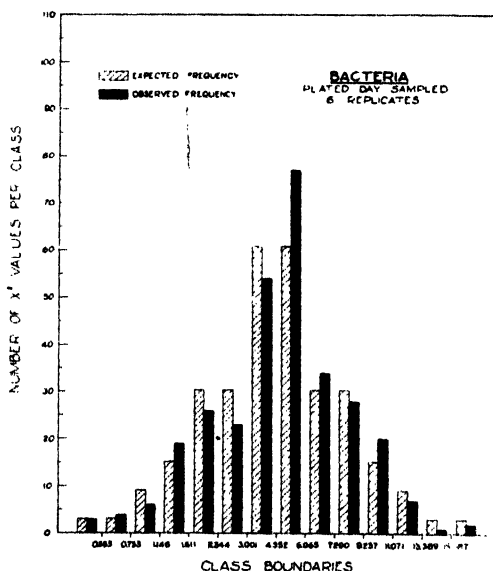
Histogram 2. χ^2 distribution on 304 samples plated immediately for counts of bacteria, six replicates.

to this distribution gives a final χ^2 value of 14.19 with a corresponding P value of approximately 0.36.   This finding was confirmed on 100 samples plated in four replicates, which yielded a final χ^2 value of 7.82 with a corresponding P value of about 0.85.

These results show that the distributions of χ^2 values conform to expectancy, and indicate clearly that the discrepancy in the distribution of χ^2 values resulting from abnormal variation among counts of replicate plates from one dilution may be avoided by proceeding with the laboratory technique of diluting and plating within a few hours after sampling.

Further evidence on the effects of holding samples of soil in the laboratory on the abnormality of the distribution of χ^2 values is shown in the data on four series of counts on samples held for different periods.   In this experiment the samples were not plated primarily to study the distribution of χ^2 values, and consequently the counts at the various ages are not on the same samples. However, they represent samples taken from the same field, plated by the same technique, covering the same period of the year (July 21 to Sept. 1, 1938), and may be considered comparable.   Each series is on 88 samples.   The first series represents samples plated in six replicates from one dilution on the afternoon of the day obtained; the second, plated in four replicates one day after breaking up; the third, in six replicates after holding five to six days; and the fourth, likewise in six replicates after eight to thirteen days. Since for given P values the χ^2 values for the class boundaries differ with the degrees of freedom, the data in the goodness-of-fit test are presented on the basis of the P value distribution, but with arbitrary numbers designating classes.

The distributions for these 352 samples are presented in Histogram 3. The goodness-of-fit test gives final χ^2 and P values of 9.62 and 0.73 for the samples plated within three to six hours; 93.86 and less than 0.01 for the samples plated after holding one day; 233.19 and less than 0.01 for the samples held five to six days; and 405.28 and less than 0.01 for the samples held eight to thirteen days.   The gradual rising of the final χ^2 values indicates a progressive increase in the degree of abnormality and suggests that the factors responsible become more marked as the time the samples are held in the laboratory is prolonged.

(b) Certain Types of Bacteria and Fungi on Plates for Bacterial Counts

During the first two years of this study, the abnormal count on one or more plates in a set frequently appeared to be associated with the presence of certain types of bacteria or fungi on the plates.   In an attempt to determine whether these types of organisms are associated with the abnormal distribution of χ^2 values, their presence was recorded on the plates prepared in 1938.   This was done on 468 samples, representing 1872 plates.   The identification and recording of these types were carried out before the plates were counted, in order not to look for their presence only on plates with low counts.   These were considered in relation to the distribution of χ^2 values.
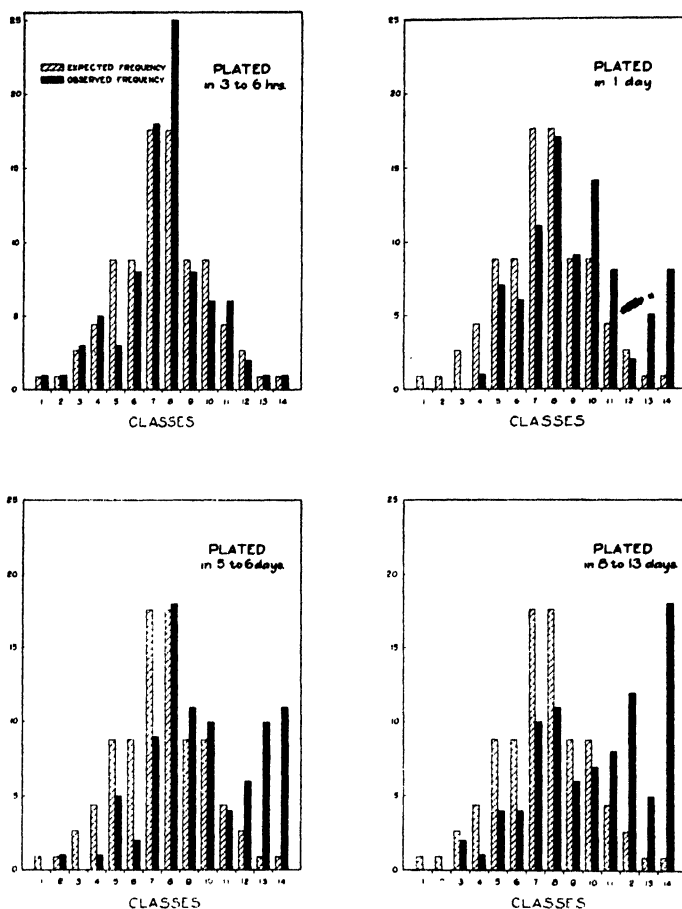
HISTOGRAM 3. χ^2 *distribution on 352 samples held varying times for counts of bacteria.*

RESULTS

The presence of an excess of pin-point colonies was noted on one or more plates from 23 samples; of these, 22 had χ^2 values with a P value lower than 0.01.   Cultures of the order Mucorales occurred 51 times.   Most of these were *Rhizopus nigricans*, but not all.   Of these, 29 sets had abnormally high χ^2 values.   *Fusarium* spp. and *Alternaria* spp. appeared in 109 samples. These were spread over the χ^2 class value range.   The same was true of spreader types of bacterial colonies, which were recorded on 91 sets.   The remaining 194 had colonies of various genera, including *Penicillium*, *Aspergillus*, *Cephalosporium*, *Cladosporium*, *Cylindrocarpon*, *Monotospora*, *Phoma*, *Trichoderma*, *Verticillium* and others occurring rarely which did not appear in one class more often than in another, or were free from unusual colonies.

The data are presented graphically in Histogram 4.   In order to simplify the presentation of these data, the term "miscellaneous" included the 194 sets

mentioned above; and *Fusarium* spp. and *Alternaria* spp. were grouped to-
gether.   Further, as plates in one set frequently had more than one type of
colony recorded, they were distributed arbitrarily in classes in the following
order of preference; pin-points, *Mucorales*, *Fusarium* and *Alternaria*, spreaders
and miscellaneous.   For example, a set with a *Mucorales* and a *Penicillium*
was placed in the *Mucorales* class only.
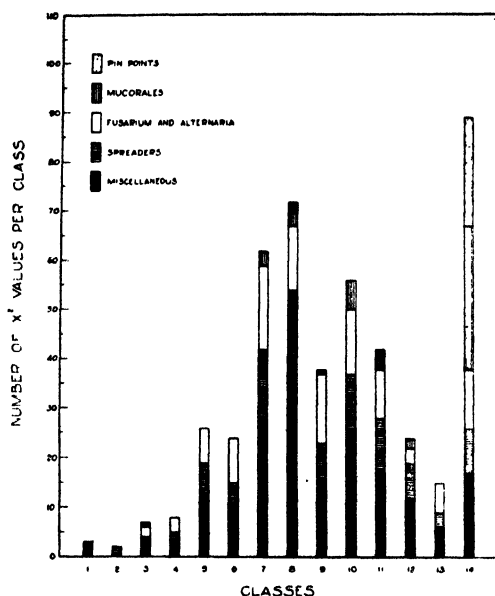
HISTOGRAM 4. χ^2 *distribution on 468 samples for counts of bacteria, 1938, showing the
effect of certain types of bacteria and fungi.*

These findings indicate that there is sound reason for rejecting plates that
have a large number of pin-point colonies or are overgrown with *Rhizopus
nigricans*, particularly if the plates concerned differ in count appreciably from
others in the set.   Had this been done in this analysis, the number of sets
having abnormally high χ^2 values would have been reduced more than one-
half.   There still remain too many sets with high χ^2 values, the cause of which
is not suggested.

(c) The Presence of Actinomyces on Plates for Bacterial Counts

The counts reported as bacterial counts in this presentation include the
actinomyces group.   Since actinomyces may be distinguished from bacterial
colonies, an attempt was made to determine whether the discrepancy in the
distribution of χ^2 values for the total count was characteristic of both groups.
During 1937, actinomyces were counted after incubation for 12 days.   The
counts in 1938 were made at eight days at the time of making the total counts,
so as to provide three sets of data for each plate, namely, the total count,
bacteria only, and actinomyces.

RESULTS

The goodness-of-fit test applied to the distribution on 483 samples investigated in 1937 gives a final χ^2 value of 32.21 and a P value slightly below 0.01. The 468 samples studied in 1938 give final χ^2 and P values of 14.51 and 0.34, respectively. The finding for 1938 appears in Histogram 5.
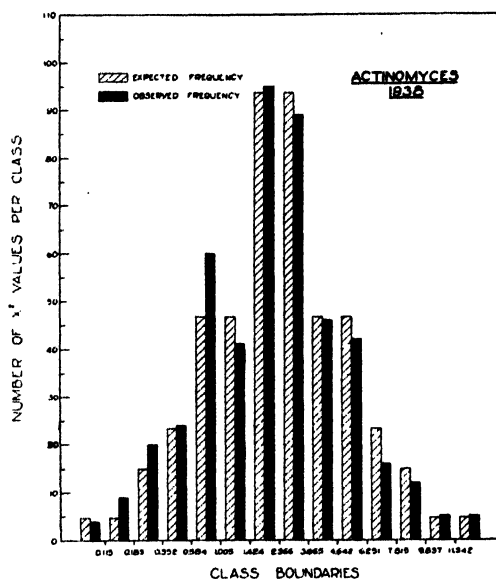
HISTOGRAM 5. *χ^2 distribution on 468 samples for counts of actinomyces, 1938.*

The counts of bacteria only, or the total count less the actinomyces, give a distribution with a final χ^2 value of 1927.43.

These results indicate that the discrepancy in the distribution of χ^2 values for the total count is due to variation among numbers of bacterial colonies rather than among numbers of the actinomyces. The factors causing the discrepancy influence the development of bacteria on certain plates, but apparently do not affect the actinomyces growth on this medium.

(d) Moisture Content of Sample Plated

Moisture tests were run on 1476 samples before plating. These varied appreciably, even among samples obtained at one time from adjoining plots. As moisture content might influence the factor or factors inducing the abnormal variation among counts on samples held in the laboratory, percentage moisture was correlated with P values corresponding to the χ^2 values obtained. Moisture readings were classed on a unit basis from 13 to 49. The P values were divided into 20 classes with an interval of 0.05 ranging from a P of 1.00 to a P of 0.00. Since the χ^2 values for all the P values required were not given in Fisher's table (11), and interpolation on a linear basis did not appear to be accurate, the figures given in this table were graphed on squared paper

on a scale large enough that the χ^2 and P values could be read to the second decimal. The χ^2 values for the missing P values were taken from the graph. A correlation surface for percentage moisture and χ^2 values was prepared from the data for each year and a correlation coefficient calculated for each.

Results

The data for 1936 give a correlation coefficient of 0.0836; for 1937, a coefficient of 0.0444; and for 1938, of 0.0697. The t test applied to the correlation coefficients gives t values of 1.87, 0.996, and 1.51 respectively. When n is taken as infinity the 5% level of significance has a t value of 1.96, and the 1%, a value of 2.58. These results may be accepted as indicating the absence of a significant correlation between moisture and χ^2 values. Consequently, moisture does not influence the distribution of χ^2 values obtained from sets of counts of bacteria on replicate plates from one dilution of soil.

III. The Accuracy of the Mean Count of Four Replicate Plates from One Dilution: Fungi

Method of Procedure

The samples referred to in Section I were plated for fungi at the same time as for bacteria. The 1 : 5000 dilution was used on all samples. The number of colonies ranged from 5 to 50, with most of the counts midway between these limits. Czapek's (12) medium was used. It was acidified by the addition of 0.5 ml. of lactic acid (10 ml. of 85% U.S.P.X. made to 100 ml. with distilled water) to 100 ml. of medium after the final tempering. Counts were made after four days' incubation at 25 to 28° C. The data were submitted to the mathematical tests applied to the bacterial counts.

Results

The goodness-of-fit test on the χ^2 distribution for counts of fungi on 468 samples investigated in 1936 gives a final χ^2 value of 32.32 and a P value slightly below 0.01. A better fit was observed on 498 samples plated in 1937; and on 467 samples in 1938. These two P values are 0.63 and 0.18. The finding for 1938 is shown in Histogram 6.

These results show a close agreement between the actual and expected distribution of χ^2 values and indicate that the mean count of four replicate plates may be used to estimate the number of fungi in the dilution plated. This is a confirmation of the finding of Jensen (17), but on a larger number of samples. It is recognized that the count may represent spores and pieces of mycelium and that the technique does not distinguish between them. Nevertheless, there should not be serious objection to the use of data obtained by this technique in comparing the numbers of potential fungi in different dilutions.
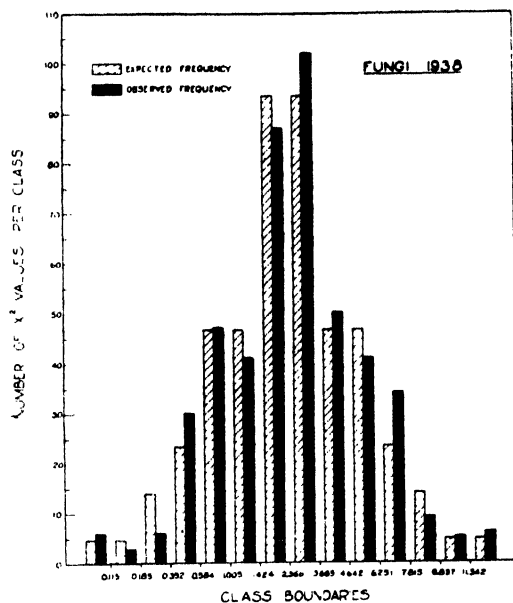
HISTOGRAM 6. χ^2 *distribution on 467 samples for counts of fungi, 1938.*

Discussion

The application of proven mathematical formulae to test the accuracy of data obtained by the soil bacteriologist requires little justification. The laboriousness of the technique involved in obtaining counts of micro-organisms by any method demands the use of every test that will verify the validity of the result or lessen the routine labour of the undertaking. The bacteriologist who makes an estimate of the numbers of fungi or bacteria in soil, does so for the purpose of relating the estimate to some condition or treatment. If his estimate is accurate, some practical advance may be made in an understanding of the intricacies of the complex medium with which he deals. If it is not, his findings retard rather than advance the science.

The most important result concerning the distribution of χ^2 values for sets of counts of bacteria has to do with the time the sample is held in the laboratory before plating. A sample plated on the day it is taken from the field yields counts that may be accepted as satisfactory for the dilution plated. The counts on samples held one day or more give χ^2 values that have abnormal distributions, similar to those presented by Harmsen and Verweel (16), who did not make clear how long their samples were held.

There still remains to be explained the cause of the abnormal distributions of χ^2 values from samples that are held before plating. Two conditions appear to be associated with an excess of high χ^2 values: a large number of pin-point colonies on one or more plates in a set, and large spreading colonies of the *Mucorales* on one or more plates. In this connection, the data presented

justify the exclusion of counts from such plates in a set. On the other hand, there is ample evidence in the data presented that certain factors bear no relation to the problem. The samples providing the good fit in the distribution of χ^2 values were obtained from the same plots as those that give the abnormal distribution. They were plated by the same technique, pertaining to diluting, pipetting, pouring, incubating, and counting, and by the same operators. The medium was the same. The counts giving a good fit were obtained over part of the same season as those that did not. This would appear to eliminate the source of soil, laboratory technique, medium and season as factors contributing to the phenomenon. The correlation data on moisture and χ^2 values show clearly that moisture content is not associated with χ^2 values. However, epidemics of high χ^2 values have been reported (9). The data presented in this study indicate that at certain samplings a larger number of high χ^2 values occur; particularly early in the summer. These could be accounted for, primarily, by the presence of pin-point colonies or excessive numbers of *Mucorales* on the sets. In fact, at all but one of the 41 platings of 36 samples in the experiments dealing with samples held one day, there is an excessive number of high χ^2 values. Further, the data for the summer of 1938 show that the causal factor does not affect the counts of actinomyces on the plates yielding the excessive numbers of high χ^2 values for counts of bacteria.

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COMPARATIVE DEVELOPMENT OF TWO WHEAT VARIETIES UNDER VARYING MOISTURE SUPPLY[1]

By J. W. HOPKINS[2]

Abstract

Two varieties of wheat, Lutescens and a cross of Reward × Caesium, were both grown in the greenhouse at four levels of soil moisture supply. The plants produced were harvested individually and subjected to structural counts, measurements, and weighings designed to illustrate certain components of growth and yield.

Significant effects of moisture supply on number, height, and weight of shoots, number of fertile heads, and weight, size, and nitrogen content of grain per plant and per shoot, differing in some cases in the two varieties, were demonstrable. The relation between the production of straw and fruiting parts per tiller also seemed to be a varietal characteristic. It is suggested that observations of this type, under field plot conditions, on a relatively small number of plants grown under controlled moisture supply, might provide useful information respecting the adaptability of different varieties of plants.

Analysis of agricultural yield through studies of the growth and development of crop plants is a problem which has received the intermittent attention of agronomists for some time. Balls and Holton in Egypt (1, 2), Engledow and his successive co-workers in England (3–5), Tincker and Jones in Wales (10), Smith in Australia (9), Immer and Stevenson in the United States (8), and Goulden and Elders in this country (6) are some of those who have dealt with one aspect or another of this subject. It would seem that information of this sort might find some application in both the genetical and agronomic phases of drought investigations, as well as in the field of crop estimation and forecasting (11). Accordingly, the small preliminary trial now to be described was conducted in the greenhouse of the National Research Laboratories, in order to provide some indications of the possibilities in this connection.

Experimental

Two varieties of spring wheat believed to differ in drought resistance were used, namely Lutescens and a cross of Reward × Caesium. Seed of these was kindly supplied by Prof. K. W. Neatby of the University of Alberta. The procedure was to grow all the experimental plants under uniform conditions until just after the initiation of tillering, when four levels of soil moisture

were imposed and maintained until maturity.   Individual plants were grown
in steam-sterilized soil in 6-in. pots, each combination of variety and treatment
being replicated six times.   In order to reduce errors due to positional effects,
the pots were arranged on the greenhouse bench in six randomized blocks,
each block comprising one representative of each variety and treatment. The
four soil moisture levels adopted were 30%, 27.5%, 25% and 22.5% res-
pectively.   These may be thought rather high for use with drought-resistant
varieties, but it should be explained that these figures refer to the level to
which the soil moisture was adjusted by weighing every three days, so that
the average moisture available would in each case be below the figure indicated.
Furthermore, the soil used, being a good quality potting loam, had quite a
high moisture-holding capacity.

Seeding took place in January, and harvesting early in June, 1938.

Results

Each plant was harvested separately and subjected to certain structural
counts, measurements and weighings.   The results of these are described in
the following paragraphs.

Table I shows the average number of shoots, heads and grain-bearing heads,
the average weight of culms, of heads and of grain, and the average number
and size of kernels per plant produced by the two varieties at the different
moisture levels.   It will be seen that the imposed differences in moisture supply
had an effect on the production of secondary tillers, but that at all four
moisture levels Reward × Caesium produced a consistently higher number of
shoots per plant than did Lutescens.   The situation in the case of the total
number of heads produced is very similar, Reward × Caesium showing a
significant excess at three of the four moisture levels.   This variety likewise
produced a consistently higher number of grain-bearing heads per plant.

The effect of moisture supply upon the production of straw is quite clear-
cut, both varieties behaving very similarly in this respect and the differences
between them being negligible.   On the other hand, the weight of heads and
of grain produced by Lutescens tends to be greater at high as well as at low
moistures.   There is no clear-cut distinction between the varieties in respect
of the average number of kernels produced per plant, but the average weight
per kernel is consistently higher in the case of Lutescens.

The manner in which the foregoing gross results were arrived at by the plant
may now be considered in a little further detail.   Table II shows certain charac-
teristics of the average plant of each variety produced under the four moisture
regimes.   The influence of decreasing moisture supply in reducing culm length,
particularly in the secondary tillers, is quite obvious.   Effects on weight of
head and on grain yield are also evident.   In both varieties the yield from
the first and second tillers is maintained fairly well throughout the moisture
range, the third and fourth tillers show marked declines, and in the case of
Lutescens the fifth disappears entirely.   This is probably a practical advantage,
since the production of a certain number of small and low-grade kernels is

TABLE I

AVERAGE STRUCTURAL COUNTS AND WEIGHTS PER PLANT

Average	30% soil moisture		27.5% soil moisture		25% soil moisture		22.5% soil moisture	
	Rew. × Caes.	Lutescens	Rew. × Caes.	Lutescens	Rew. × Caes.	Lutescens	Rew. × Caes.	Lutescens
No. of shoots	5.8	4.8	5.0	4.7	5.2	4.8	4.3	3.5
No. of heads	5.0	4.3	4.5	4.0	4.5	4.7	4.2	3.3
No. of grain-bearing heads	4.3	4.0	4.2	3.8	3.8	3.7	3.5	3.0
Wt. of culms, gm.	1.9	1.9	1.6	1.5	1.2	1.2	0.9	0.8
Wt. of heads, gm.	4.1	4.7	4.0	3.9	3.0	3.7	2.7	2.8
Wt. of grain, gm.	3.1	3.8	3.0	3.0	2.2	2.9	2.0	2.2
No. of kernels	107	109	103	93	75	94	71	62
Wt. per 1000 kernels, gm.	28.9	35.1	29.4	32.8	29.3	30.8	28.3	34.4

TABLE II

AVERAGE STRUCTURAL MEASUREMENTS PER TILLER

Average	Tiller No.	30% soil moisture		27.5% soil moisture		25% soil moisture		22.5% soil moisture	
		Rew. × Caes.	Lutescens	Rew. × Caes.	Lutescens	Rew. × Caes.	Lutescens	Rew. × Caes.	Lutescens
Length of culm, cm.	1	74.5	77.8	79.0	72.2	66.3	67.3	68.4	64.2
	2	71.4	69.4	70.8	65.8	59.6	60.6	54.6	53.1
	3	62.6	63.0	62.7	60.6	52.7	55.3	43.6	43.3
	4	48.8	47.6	52.1	41.4	39.4	44.8	35.1	10.1
	5	30.6	22.7	24.0	6.3	17.8	16.0	5.0	0.0
Wt. of head, gm.	1	1.07	1.44	1.06	1.21	0.82	1.17	0.93	1.22
	2	1.10	1.29	1.06	1.12	0.87	0.97	0.94	0.87
	3	1.04	1.12	0.97	1.07	0.76	0.95	0.58	0.55
	4	0.56	0.75	0.62	0.50	0.31	0.52	0.23	0.04
	5	0.23	0.10	0.16	0.01	0.08	0.08	0.01	0.00
Wt. of grain, gm.	1	0.81	1.26	0.85	1.01	0.64	0.95	0.76	1.00
	2	0.82	0.98	0.80	0.96	0.72	0.77	0.68	0.73
	3	0.81	0.93	0.78	0.83	0.64	0.77	0.52	0.48
	4	0.36	0.51	0.51	0.34	0.16	0.38	0.14	0.05
	5	0.12	0.04	0.09	0.00	0.03	0.00	0.00	0.00
No. of kernels	1	28	36	30	30	22	28	28	29
	2	30	25	27	29	24	27	24	21
	3	28	28	26	25	22	25	14	12
	4	14	15	17	10	6	12	4	1
	5	5	2	3	0	1	0	0	0

thereby avoided. The additional grain yield of Lutescens, noted in Table I, is thus mainly attributable to the superior performance of the first, second and third tillers and not, as might perhaps have been expected, to a better sustained fruiting capacity in the fourth and fifth side tillers.
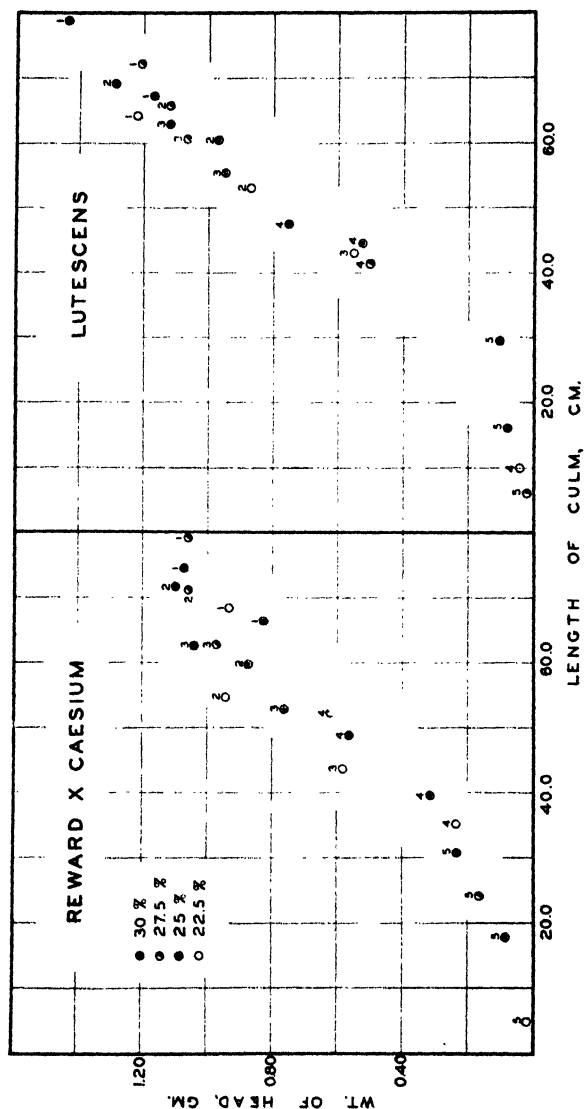
FIG. 1. Average length of culm and weight of head of successive tillers of plants grown under four different levels of soil moisture supply. Superscripts indicate first, second, third, etc., tillers.

Turning now to intra-varietal comparisons, Fig. 1 shows for each variety separately the average weight of the head plotted against the average length of culm of successive tillers of the plants produced under each condition of soil moisture supply. For the most part there is a fairly close relation between these two attributes, which is maintained under all four soil moistures. The

four values for the first tillers of Reward × Caesium, however, (which are distinguished in the diagram by the superscript 1) provide exceptions to this general tendency. It is thought that this anomaly may be due to the fact that the primordia from which these heads developed, being the first to be
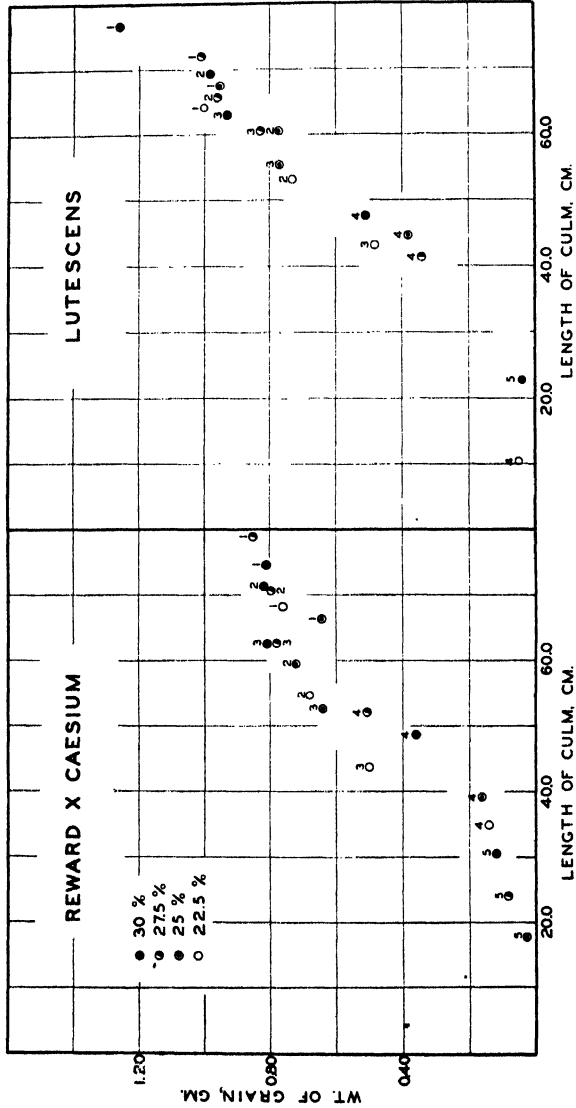
FIG. 2. Average length of culm and weight of grain of successive tillers of plants grown under four different levels of soil moisture supply. Superscripts indicate first, second, third, etc., tillers.

laid down, suffered from unfavourable growth conditions (chiefly low light intensity) in the greenhouse during the winter period. If the points in question are excluded, the correlation is appreciably improved. In the case of Lutescens, the relation as a whole is quite regular and the first tillers fit well into the general trend. It will be observed that the correlation in both cases is non-

linear, and that the curve for Lutescens rises more steeply. The point and degree of curvature, as well as the general slope, may therefore be varietal characteristics of some practical importance under drought conditions, since they are measures of the extent to which the production of fruiting parts is maintained when vegetative development, as indicated by straw length, is limited.

Fig. 2 shows a very similar relation between grain yield and culm length. The correlation is again curvilinear, and the results for all four moistures fall into the same general system, but the actual details of the relation would seem to be somewhat different for the two varieties. The curved form of the relation is suggestive of an example of the differential relative growth rates of organs, of the type made familiar by Huxley and termed by him "heterogony" (7).

This supposition receives some support when the foregoing data are re-plotted on a logarithmic scale, as is shown in Fig. 3, the approach to the required linearity being quite close, particularly in the case of Lutescens. Here also, then, there might be a possibility of distinguishing between varieties, on the basis of their relative growth gradients.
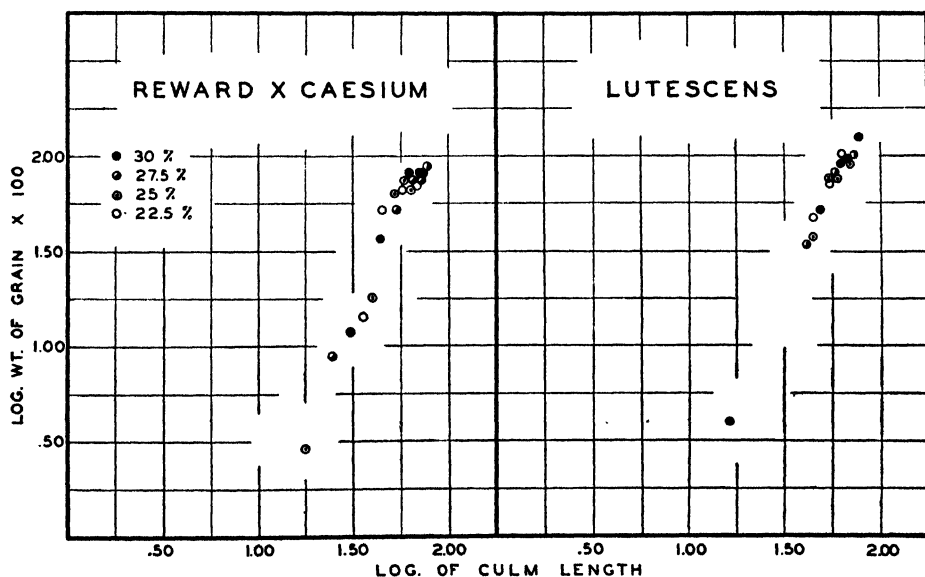
FIG. 3. *Average length of culm and weight of grain, in logarithmic units, of successive tillers of plants grown under four different levels of soil moisture.*

Differential relative growth rates may occur not only in separate organs, but also in different parts of the same organ, as is illustrated in Fig. 4. Here the average length of the top internode of the various culms of the plants at each soil moisture is plotted against the corresponding average length of the basal internodes. Once more there is a tendency towards curvilinear corre-lation, the length of the topmost internode increasing rather more than propor-

tionately with that of the basal ones.   It may also be noted that the ratio of top to basal internode length is in general higher for Reward × Caesium than for Lutescens.
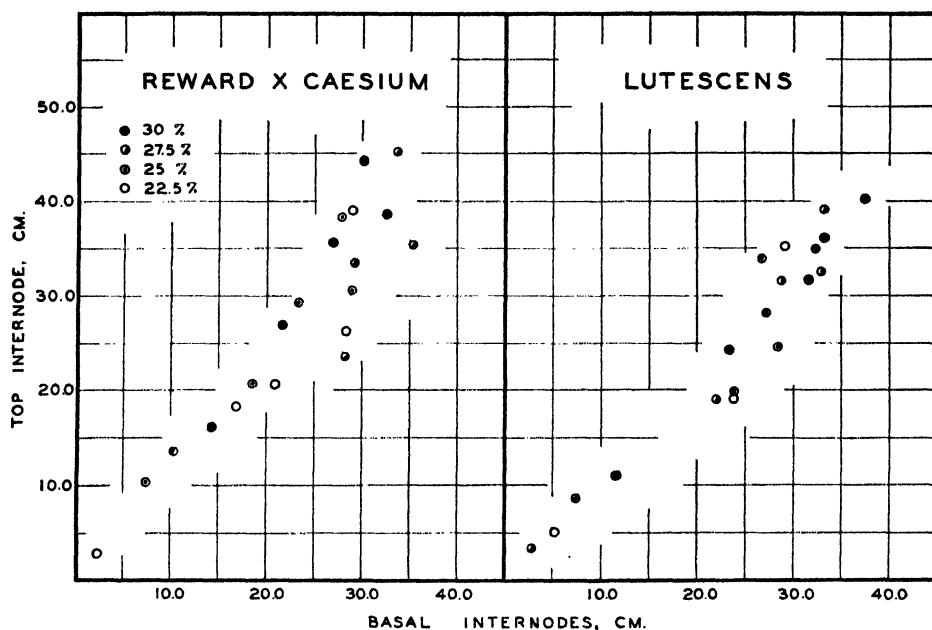
FIG. 4.   *Average length of basal and top internodes of culms of successive tillers of plants grown under four different levels of soil moisture supply.*

Whilst the general trend is quite clear, there are a number of individual irregularities, and it seemed worth while to ascertain whether these were significantly associated with grain yield.   It was found that the association between yield and total length of culm gave rise to a correlation coefficient of 0.948 in the case of Reward × Caesium and 0.972 in the case of Lutescens. In neither instance was this correlation significantly increased by considering the lengths of the upper and lower internodes separately.

Table III shows the results of nitrogen analyses, by the Kjeldahl method, of the grain from the individual heads.

Weighting the result for each head in proportion to its contribution to the total yield, the nitrogen content of both varieties clearly increases as soil moisture is withheld and yield reduced.   From the unweighted averages of the first, second, etc., tillers it is further seen that this increase is to some extent distributed over the whole plant.   In Reward × Caesium, however, the increment is more pronounced in the fourth and fifth side tillers than in the first three.   In Lutescens, on the other hand, this is not the case to nearly the same extent, since the fifth tillers of this variety, even if present, were infertile at all but the highest moisture level.   The results as a whole thus do not in all respects parallel those reported by Engledow and Wadham (5), '

TABLE III

AVERAGE NITROGEN CONTENT OF GRAIN, AS PERCENTAGE OF DRY MATTER

Average	30% soil moisture		27.5% soil moisture		25% soil moisture		22.5% soil moisture	
	Rew. × Caes.	Lutescens	Rew. × Caes.	Lutescens	Rew. × Caes.	Lutescens	Rew. × Caes.	Lutescens
Tiller No. 1	3.62	2.93	3.58	3.43	3.80	3.42	3.87	3.70
No. 2	3.54	2.84	3.54	3.17	3.73	3.41	3.54	3.64
No. 3	3.38	2.74	3.27	3.25	3.78	3.22	3.70	3.50
No. 4	2.94	2.75	3.26	3.39	4.04	3.35	4.20	3.93
No. 5	3.30	2.64	3.91	—	4.49	—	—	—
Whole plant	3.37	2.81	3.37	3.23	3.72	3.33	3.73	3.62

who found a progressive increase in the nitrogen content of the grain from successive side tillers of barley plants grown under English field conditions.

In conclusion, it should perhaps be once again pointed out that the purpose of this study was purely exploratory, and it is not suggested that the present results, secured under winter greenhouse conditions, would be duplicated in all respects in the field. It does seem reasonable to infer, however, that observations of this type, under field plot conditions, of a relatively small number of plants grown under controlled moisture supply, might provide useful information respecting the adaptability of different varieties of plants, and also respecting the morphological aspects of drought resistance in general.

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THE ACCURACY OF THE PLATING METHOD FOR ESTIMATING THE NUMBERS OF BACTERIA AND FUNGI FROM ONE DILUTION AND FROM ONE ALIQUOT OF A LABORATORY SAMPLE OF SOIL[1]

By NORMAN JAMES[2] AND MARJORIE L. SUTHERLAND[3]

Abstract

Six 25-gm. aliquots taken from a well-mixed laboratory sample were plated, each in six replicate dilutions with four replicate plates from each dilution, for counts of fungi. The analysis of variance shows that there are not significant differences in counts of fungi among replicate dilutions from one aliquot, but that there are among aliquots from one laboratory sample. Each replicate dilution was raised and the final dilutions plated in four replicates for counts of bacteria. The data for bacterial counts show significant differences among dilutions from one aliquot, but not among aliquot samples.

In a second experiment, one 25-gm. aliquot taken from a sample was diluted 1 : 10 and another was diluted 1 : 50. Each original dilution was raised to 1 : 5,000 in 11 replicate dilutions, which were plated in four replicates for fungi. The experiment was repeated 10 times. In this case, the data show that the 1 : 10 method of making the original dilution yields significant differences among the final dilutions and that the 1 : 50 system, which reached 1 : 5,000 in one transfer, is preferable. Each dilution was raised to 1 : 500,000 and the final dilutions were plated for bacteria in six replicates. The analysis shows that the 1 : 10 method is not reliable because of significant differences among dilutions and that the 1 : 50 method is preferable, although failing to reduce the differences to insignificance.

The 1 : 50 and 1 : 100 systems of making the original dilution were compared in Experiment 3, as well as differences among aliquot samples. A fresh sample was plated in five aliquots for each system, each aliquot in ten replicate dilutions and each dilution in four replicate plates for bacteria. The 1 : 50 system again shows significant differences among dilutions and the 1 : 100 system is not preferable. Likewise, there are significant differences among aliquot samples in each case.

In Experiment 4 all dilutions were raised from 1 : 50 original dilutions. Each trial consisted of six aliquots, raised in six replicate series of dilutions and plated in six replicate plates from each final dilution. This was repeated four times for fungal counts and six times for counts of bacteria. The analysis again shows that for fungal counts differences among dilutions are not significant, while for bacterial counts they are. Again, there are significant differences for aliquot samples in the case of both fungal and bacterial counts.

In Experiments 2, 3, and 4, the plating, pouring, piling of plates in the incubator and counting of plates were carried out in one order. The analysis shows that none of these practices adds anything significant to the error of plating.

As the errors of the sample used and of the dilution plated are significant, reliable information on the counts of bacteria, actinomyces, or fungi in a laboratory sample is not obtained by the usual procedures with one 25-gm. sample and one final dilution from it, regardless of the number of replicate plates made from the dilution. By the use of six aliquot samples with three replicate dilutions from each, and one plate for each dilution, the estimate would be based upon these three factors in about their proportionate weight.

Only by carefully designed experiments and the application of statistical methods to check the validity of the results obtained can progress be made in developing the plate method of counting bacteria or fungi in soil to a stage where it may be used for practical application to the problems of agriculture.

Introduction

At the conclusion of the plating of 504 samples of soil by the methods described in a previous paper (3), it seemed apparent that counts on samples from plots receiving one cultural or fertilizer treatment varied appreciably and did not show a consistent relation to those from plots treated differently. The 36 samples at each sampling represented six treatments replicated six times. Each plot was sampled seven times during the season. On the assumption that 42 replicate samples from plots receiving a given treatment would yield counts differing from 42 replications from similar plots that were not treated, it seemed advisable to consider the probability of there being a weakness in the technique of dilution and sampling in the laboratory. A series of replicate dilutions from one sample could be made and a statistical test applied to the data to determine whether the result from one dilution gives a reliable estimate of the population in the sample. Similarly, a series of aliquot samples could be studied to determine whether one sample is sufficient to give a reliable estimate of the population in the large sample brought to the laboratory. The analysis of variance was considered for this study.

In view of the fact that counts of bacteria and fungi in general follow the Poisson series, it may be questioned whether the analysis of variance should be applied to such data. This subject has been considered by Cochran (2), who makes the following statement, "So long as treatment responses are of the order of 50% or under, and the standard error per plot is under 12%, there can be little wrong with the use of analysis of variance, no matter what form the data analyzed may take". When the analysis is applied to data obtained for the improvement of technique there appears to be no reason for making adjustments of the data before the analysis is made. The variation among means for dilutions in the data presented frequently exceeds the 50% limit in the case of counts of bacteria plated from dilutions raised from the 1 : 10 original dilutions. When the technique is improved by using the 1 : 50 system of making the original dilutions the variation in means is below 40% in the majority of cases. The standard error for the units analyzed for variance among dilutions is of the order of about 15% for counts of bacteria and 20% for fungal counts.

The variation among replicate counts of fungi has been considered (3), and has been found to conform to expectancy on the basis of random sampling. This variation among parallel plates may be accepted arbitrarily as the error in the analysis of variance. If the variance among dilutions is larger than the error variance, there is sound reason for considering this to be a source of serious error in the method of obtaining counts by the plate technique. If, on the contrary, the variance is about proportional to the error, this may be accepted as indicating that the system of diluting adds nothing to the error. The same holds true for the aliquot sample used in making the dilution.

The case for bacteria is more complicated. The distribution of χ^2 values in the earlier studies does not conform to expectancy, and the error is large

as shown by an excess of high χ^2 values.   Significant variation among dilutions or aliquot samples is even more serious than in the case of fungi, since it is compared with an error that may be larger.   Fortunately, in the later studies this error is reduced and the effects of dilutions and aliquot samples are shown with more precision.

Experiment 1

Method of Procedure

A sample held in the laboratory one week was sieved and mixed thoroughly. Six 25-gm. aliquots were transferred to six 240-ml. dilution blanks and shaken as described previously (3).   Each 1 : 10 dilution, obtained in this manner, was given a vigorous shaking by hand immediately before a transfer to each of six higher dilutions was made.   These replicate dilutions were raised to make 36 final 1 : 5,000 dilutions representing six aliquots from one sample. Each dilution was plated in four replicates for counts of fungi.

Each dilution was raised to 1 : 200,000 and plated for counts of bacteria. Owing to a mishap one plate was lost.   Accordingly, the data represent 30 dilutions from five aliquot samples.

Results

In the analysis of variance for each aliquot sample there are 5 degrees of freedom for dilution variance and 18 for error variance.   For 18 degrees of freedom (the smaller mean square) and 5 degrees of freedom (the greater mean square), the 5% level of significance has an F value of 2.77 (4).   The six aliquot samples have F values for dilutions as follows: 0.649, 1.20, 1.53, 1.36, 0.740 and 0.415.   These are all less than the 5% level of significance.

The complete analysis of variance for the six aliquot samples follows in Table I.

TABLE I

COMPLETE ANALYSIS OF VARIANCE—FUNGI

	Sum of squares	D.f.	Mean square	F	5% level
Total	3928.000	143			
Aliquot samples	715.167	5	143.0332	6.00	2.30
Dilutions	636.833	30	21.2278	.89	1.56
Error	2576.000	108	23.8518		

These results suggest that for fungal counts the variance among dilutions does not add to the error of plating, while that among aliquot samples introduces serious error in the method.

A similar analysis of the data for counts of bacteria on five aliquot samples shows F values for dilutions as follows: 4.04, 5.52, 1.46, 0.435 and 2.14.

Two of these are above the 5% level of significance. The complete analysis for the five aliquots follows in Table II.

TABLE II

COMPLETE ANALYSIS OF VARIANCE—BACTERIA

	Sum of squares	D.f.	Mean square	F	5% level
Total	13621.9667	119			
Aliquot samples	520.0921	4	130.0230	1.61	2.47
Dilutions	5840.8746	25	233.6350	2.90	1.63
Error	7261.0000	90	80.6778		

This analysis shows that for bacterial counts the variance among dilutions is significant, while that among aliquot samples is not. Again, attention is drawn to the fact that the error for counts of bacteria is large. Accordingly, the effect of variation among dilutions undoubtedly is more serious than the result shows. Further, the finding for aliquot samples may be affected seriously by the errors of replicate plates and dilutions. These errors must be reduced to a minimum before valid comparisons for dilutions or aliquot samples can be made.

Experiment 2

This experiment was undertaken before the error of replicate plates for counts of bacteria was under proper control. Accordingly, the error variance may be large and the effect of variation among dilutions may be more serious than is shown in the data. Three points appeared worthy of further study; firstly, to substantiate the finding concerning dilutions for fungal and bacterial counts on a more comprehensive scale; secondly, to determine whether the use of a larger quantity of water in the original dilution would lessen the discrepancy among counts from different dilutions from one aliquot sample; and thirdly, to ascertain whether there was systematic error in plating. The last problem seemed important from the standpoint of reference to many minor points in technique such as: the order of plating, the order of piling plates in the incubator and the order of counting plates.

METHOD OF PROCEDURE

The soil samples were held in the laboratory up to seven days before plating. In each trial the sample was sieved and mixed thoroughly. A 25-gm. aliquot was suspended in 240 ml. of sterile water. From this 1 : 10 dilution 11 replicate series of dilutions were made, yielding 11 final 1 : 5,000 dilutions for plating for counts of fungi. Another 25-gm. sample was suspended in 1240 ml. of sterile water. From this 1 : 50 dilution 11 series of dilutions were made to give the 1 : 5,000 dilutions. Each final dilution was plated in four

replicates. Thus each trial gave 44 plates from 11 dilutions of one sample using the 1 : 10 system of making the original dilution, and another 44 plates using the 1 : 50 system. The experiment was replicated ten times.

The dilutions used for making fungal counts were raised to 1 : 500,000. These were plated for counts of bacteria in six replicates. This gave 66 plates for bacterial counts for each system of making the original dilutions. Likewise, this procedure was repeated ten times.

To introduce a large interaction variance between dilutions and replicate plates, if possible, the plates were prepared, poured, piled in the incubator and counted in systematic order. In the analysis, the interaction variance is based on columns, obtained by recording the counts in order. The number of degrees of freedom for interaction is the product of the number of degrees of freedom for dilutions and that for parallel sets of plates.

RESULTS

In the analysis of variance for each sample there are 10 degrees of freedom for dilution variance and 33 for error. For 33 and 10 degrees of freedom the F values at the 20 and 5% levels of significance are 1.465 and 2.153, respectively.

The 1 : 10 method of making the original dilutions for counts of fungi gives the following F values for dilutions in the ten replications: 1.49, 2.30, 0.880, 0.996, 1.61, 1.58, 1.59, 2.35, 0.726 and 0.648. These may be distributed and compared with the theoretical distribution as follows:

P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	5	3	2
Theoretical	8	1.5	.5

The 1 : 50 method of making the original dilutions gives the following F values for dilutions: 0.939, 0.718, 1.39, 1.98, 0.546, 2.01, 0.803, 0.655, 2.22 and 0.694. Their distribution follows:

P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	7	2	1
Theoretical	8	1.5	.5

These results appear to suggest that there are significant differences among dilutions in counts of fungi and to indicate that the 1 : 50 method of making the original dilutions is preferable.

As the experiments that follow yield further information on the interaction variance in plating, this part of the data will be considered later.

In the analysis on bacterial counts there are 10 degrees of freedom for dilution variance and 55 for error variance. For 55 and 10 degrees of freedom the F values at the 20% and 5% levels of significance are 1.42 and 2.00, respectively.

The ten F values obtained by the 1 : 10 method of making the original dilutions follow: 3.53, 3.07, 2.94, 4.16, 2.63, 2.65, 2.73, 2.15, 3.75 and 1.31, with this distribution:

P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	1	0	9
Theoretical	8	1.5	.5

The 1 : 50 method of making the original dilutions gives the following F values for dilutions: 1.00, 2.00, 1.28, 1.78, 0.943, 2.49, 1.68, 1.45, 1.67 and 1.72, distributed as:

P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	4	4	2
Theoretical	8	1.5	.5

Here, as with fungal counts, the results for the 1 : 50 method show an error for dilutions beyond that for replicate plates, but indicate a marked reduction in variation due to dilutions.

Experiment 3

The 1 : 50 method of making the original dilutions appeared to reduce the differences among dilutions. Consequently, this experiment was designed to confirm the finding, to determine the effect of further increasing the proportion of water in the original dilution, and to measure differences in aliquot samples by the improved technique.

METHOD OF PROCEDURE

The sample used in this study was plated on the day it was taken from the field. The counts of bacteria only were considered. The 1 : 50 and 1 : 100 methods of making the original dilutions were compared in five replications. Each consisted of ten replicate dilutions with four plates from each. This gave 200 plates for each method of making the original dilution.

RESULTS

In this experiment there are 9 degrees of freedom for dilution variance and 30 for error. For 30 and 9 degrees of freedom the F values at the 20 and 5%

levels of significance are 1.482 and 2.225 respectively. The 1 : 50 method of making the original dilutions gives F values for dilutions of 2.37, 0.901, 1.05, 2.11 and 2.03, distributed as follows:

P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	2	2	1
Theoretical	4	.75	.25

The 1 : 100 method of making the original dilutions gives F values of 1.26, 1.11, 2.87, 3.27 and 3.83, with the following distribution:

P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	2	0	3
Theoretical	4	.75	.25

This result suggests that the 1 : 100 method of making the original dilutions is not an improvement over the 1 : 50 method in so far as the error caused by dilution differences is concerned.

For 150 and 4 degrees of freedom for aliquot samples, the 1% level of significance has an F value of 3.44. In the 1 : 50 method, the F value for aliquot samples is 7.80; and in the 1 : 100 method, 9.64. These results indicate significant differences in counts of bacteria among aliquots taken from one sample. In this case the error of replicate plates was reduced to that of random sampling by the use of a fresh sample. Likewise, the error of dilutions was lessened appreciably by the system of diluting.

Experiment 4

This experiment was designed primarily to determine whether there are significant differences in counts of fungi and of bacteria among aliquot samples, and to obtain more data on dilution variance and on interaction variance in plating from samples of soil plated on the day obtained from the field.

METHOD OF PROCEDURE

All plating was carried out on dilutions prepared from 1 : 50 original dilutions. Each trial consisted of six aliquot samples. Each aliquot sample was plated in six dilutions with six replicate plates from each. This was repeated four times for fungal counts and six times for counts of bacteria.

RESULTS

For 180 and 5 degrees of freedom for aliquot samples, the 1% level of significance has an F value of 3.122. The data for fungi give F values of 78.48, 2.09, 53.14 and 3.49. For bacteria the F values for aliquot samples are

15.46, 2.16, 6.21, 5.97, 27.16 and 19.11. From these figures it appears obvious that aliquot samples introduce a large and serious error in estimating the populations of fungi and bacteria in a sample brought to the laboratory.

When the data for dilutions are considered, the findings of previous experiments are confirmed. In the case of fungi there are 30 and 5 degrees of freedom for dilutions. For these degrees of freedom, the F values at the 20 and 5% levels of significance are 1.57 and 2.53, respectively. The distribution follows:

P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	18	4	2
Theoretical	19.2	3.6	1.2

The distribution for bacterial counts is as follows:

P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	19	7	10
Theoretical	28.8	5.4	1.8

Summary of Data for Dilution Variance

The following distributions combine the dilution variance data on the 1 : 50 system of making the original dilutions for Experiments 2 and 4 in the case of fungal counts, and for Experiments 2, 3 and 4 for counts of bacteria. The degrees of freedom available for the estimation of the effect of dilutions vary in the different experiments, but the 20 and 5% points for each experiment were determined as the F values were distributed into classes in each case. This is the best evidence at our disposal in this study on the effect of dilutions, although it cannot be taken as final proof because of the small number of values involved.

The distribution of 34 F values for fungal counts is presented first:

P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	25	6	3
Theoretical	27.2	5.1	1.7

The distribution of 51 F values for counts of bacteria follows:

P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	25	13	13
Theoretical	40.8	7.65	2.55

Summary of Data for Systematic Error

As referred to in Experiment 2, a deliberate attempt was made in the last three experiments to introduce as large a systematic error as possible without interfering with the regular routine of plating. As one would expect the same sources of systematic error to affect the fungi and the bacteria alike, the two sets of data for distributions are considered together.

P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	92	18	8
Theoretical	94.4	17.7	5.9

In this attempt to introduce a systematic error it is apparent that the routine technique involved in large scale plating of soil samples for counts of fungi or bacteria adds nothing significant to the error.

Discussion

The variation between two dilutions from one aliquot sample or between two aliquots from one laboratory sample introduces a problem that does not appear to have been considered previously. It is obvious that the count obtained, however accurate it may be made by refinements of laboratory technique and replication of plates, represents merely an accurate count *on the dilution plated*. This limitation must be recognized. If the dilution plated is not representative of the sample in the laboratory or the soil in the field, the count has no value for the purpose intended.

The data show clearly that one dilution made by starting with 25 gm. of soil in 240 ml. of sterile water and raising it to the required dilution for plating does not provide a reliable sample for estimating the number of bacteria or fungi per gram in the 25 gm. of soil used. Variation in dilution blanks and in pipettes undoubtedly causes part of the error of dilutions in this study. Each 1 : 5,000 final dilution plated for fungi represented two consecutive dilutions of the original 1 : 10 dilution or one dilution of the 1 : 50 original dilution. Likewise, each final 1 : 500,000 dilution plated for bacteria and actinomyces represented three consecutive dilutions of the 1 : 10 original dilution and two only of the 1 : 50 original dilution. That is, the error of the dilution blanks and of pipettes used in transferring was in the ratio of 2 to 1 for fungal counts and 3 to 2 for bacterial counts, when the 1 : 10 system of obtaining the original dilutions is compared with the 1 : 50 system. The 1 : 50 system of making the original dilution proves to be superior in that it reduces differences between replicate dilutions for counts of fungi and bacteria.

An interesting comparison of the results obtained by using two consecutive dilution blanks in raising the original dilution to the one used for plating fungi and bacteria may be made. The data on analysis of variance for counts of fungi in dilutions raised from the 1 : 10 original dilutions in two consecutive

dilutions, presented in Experiment 2, show the distribution of F values in classes for F corresponding to certain P values. The theoretical number in each class is given also. A ratio may be obtained by dividing the actual by the theoretical number in each class. Similar data for counts of bacteria, in dilutions raised from the 1 : 50 original dilutions in two consecutive dilutions, may be found in the summary of data for dilution variance, and similar ratios may be obtained. The ratios follow:

	Fungi, 1 : 10 dilution			Bacteria, 1 : 50 dilution		
P	1.00–0.20	0.20–0.05	0.05–0.00	1.00–0.20	0.20–0.05	0.05–0.00
Actual	5	3	2	25	13	13
Theor.	8	1.5	.5	40.8	7.65	2.55
A/T	.625	2	4	.613	1.70	5.10

The close similarity in the ratios of actual to theoretical in the corresponding classes for the two sets of data seems to suggest that variation in dilution blanks and in pipettes is one of the main causes of the error of dilutions. When the 1 : 50 method of making the original dilution is used, one dilution blank only is required to raise to the 1 : 5,000 dilution used for plating. In the data for fungi, presented in the summary of data for dilution variance, this method reduces the effect of dilutions from that of the 1 : 10 for fungi shown above to the following:

	Fungi, 1 : 50 dilution		
P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	25	6	3
Theoretical	27.2	5.1	1.7
A/T	.9191	1.1765	1.7647

Since there is such a close agreement in the ratios of actual to theoretical for fungi and bacteria when two dilution blanks were used for raising, it is probable that a similar improvement would result if one dilution blank only were used for raising to the dilution required for plating bacteria. This would mean the use of much larger dilution blanks than is the general practice.

The possibility of variation in dilution blanks introducing error in the technique was recognized early. Accordingly, over 400 blanks selected at random after sterilization were measured in a 100-ml. burette. They were found to vary as much as $\pm 3\%$. This is a greater tolerance than the $\pm 2\%$ allowed in the Standard Methods of Milk Analysis (1). The pipettes used were uniform in type, but were not checked for tolerance.

Another cause of the error of dilutions probably is the rapid settling of the soil in the 1 : 10 dilution before the first transfer is made.   In other words, the mixture of soil in water does not represent a uniform suspension.   The relatively better result for counts of fungi may be due to the size of the fungal spores and pieces of mycelium.   These would be separated from the soil particles more readily and would remain suspended longer than soil particles, which carry the bacteria down.

The use of 1240 ml. of water to suspend 25 gm. of soil in the original dilution provides a marked improvement in technique.   The variance among dilutions for counts of fungi is reduced to insignificance by this procedure.   Accordingly, one dilution may suffice to provide an estimate of the population in the 25-gm. aliquot sample used.   This is not so for counts of bacteria.   The variance among dilutions, although reduced by this method of diluting, is still highly significant.

This being the case, there is no alternative other than to suggest the use of a 1 : 50 original dilution and the replication of dilutions, rather than the replication of plates from one final dilution.   This is shown clearly in the data for systematic error.   The variance for columns is not significant.   This means that one plate from each of six dilutions produces an estimate that does not differ significantly from that of another plate from each of the same dilutions.   The greater the number of replicate dilutions the more accurate the estimate of the population in the sample diluted.   Again, it must be realized that the result at this stage provides merely an estimate *on the 25-gm. aliquot sample* used in preparing the dilutions.

The data show conclusively that one 25-gm. aliquot sample does not provide an accurate estimate of the population of fungi or bacteria in the sample brought to the laboratory.   Further, the variation among aliquot samples is much greater than that among replicate dilutions of one aliquot sample.   Accordingly, this furnishes the most serious error in the technique. It should be given first consideration and more weight than the error of dilutions or of replicate plates.   As a suggestion, if one is to limit the study to 18 plates prepared from one laboratory sample, six aliquot samples should be plated in three replicate dilutions with one plate for each dilution.   The data presented do not justify outlining a definite procedure in this respect, but substantiate the conviction that, until some improved method of reducing the error of the aliquot sample and that of the dilution has been established, little valid information can be obtained with less labour.

The interest in systematic error rests wholly on significance.   The data show clearly that, compared to the error variance, the minor errors of the technique are not significant.   This does not lessen the necessity for carrying out the procedure in a careful and orderly manner.   At the same time, nothing practical may be expected to result from certain refinements in technique that may seem important in the plate method.   In this study the first plate prepared, poured and counted did not differ significantly from any other in the set.   The same was true of the bottom plate in the pile.

Probably the chief problem in obtaining an estimate of the population per gram of soil in a given field has not been considered in this presentation. It is obvious that an estimate of the population in a laboratory sample has little value if it cannot be related to the field soil. It is equally clear that, if the estimate on the laboratory sample is not accurate, valid information on the population cannot be obtained. On this assumption this important point has been left for later study.

Finally, the data presented appear to provide sound reason for the uncertainty about the value of the plate method referred to in a previous paper (3). Likewise, they suggest that much that has been done in an attempt to apply counts of micro-organisms to certain conditions or treatments may be worthless. Each of the above two statements is a condemnation of the method as it has been used. Neither has any relation to the method that may be developed by the application of statistical methods to prove the validity of each step in the procedure.

Acknowledgments

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THE AEROBIC DECOMPOSITION OF GLUCOSE IN PODSOL SOILS[1]

By P. H. H. Gray[2] and C. B. Taylor[3]

Abstract

The decomposition of glucose in samples from cultivated podsol soils of the Appalachian upland region of Quebec Province was effected rapidly by aerobic micro-organisms without the aid of added nitrogen. Potassium nitrate stimulated the rate of carbon dioxide production from glucose added to soil; the rate increased chiefly during the early stages while glucose was still present, and bacterial numbers were rising. Fungi developed high numbers later than the bacteria; they developed especially in soil to which glycine was added with the glucose. Biological activity was stimulated in soils in which glucose had previously been decomposed. The decomposition of the glucose appears to release other sources of available food material.

Introduction

Among the factors that have an essential bearing on the decomposition of carbonaceous residues in soil, the nature and amount of available nitrogen would appear to be of first importance. It has been found that the addition of available nitrogen, in the form of salts of ammonia or of nitric acid, or in manure, stimulates the decomposition of cellulose (2). It has also been shown that the organisms in soils that have not received such treatments are capable of decomposing cellulose, though at a much slower rate. This is usually accounted for by the existence in the soil of a small amount of some form of available nitrogen. The concentration of nitric nitrogen present in soil appears to have some effect on the amount of cellulose decomposed; in fact, the relation is masked when an excessive amount of nitrate nitrogen is added (15). The nature of the nitrogen compounds, other than ammonium salts, nitrites and nitrates, available to the organisms in soil is not known, but they are assumed to be adsorbed ammonium compounds derived from the decomposition of soil protein. Nitrate nitrogen would appear to be the most important source of nitrogen for the heterotrophic micro-organisms.

It has been established that the decomposition in soil of plant materials, such as straw, is controlled by the availability of the organic nitrogen in the material or, as it is generally stated, by the ratio of carbon to nitrogen. Material that has a high C : N ratio is decomposed more rapidly if ammonium or nitrate nitrogen is added to supply its natural deficiency in available nitrogen (1, 12, 14); that is, the C : N ratio must be lowered if microbial decomposition of the carbonaceous material is to proceed efficiently.

Organic compounds containing no nitrogen, such as decomposition products of cellulose or cellulosic material, may be a cause of the temporary removal of nitrogen and result in deficient plant growth. This is generally accepted as the reason for lowered yields after the application of straw to soil, or its use as a mulch. Such treatments may, however, ultimately result in gains through the use of the carbonaceous material by nitrogen-fixing bacteria, whose cells will in time become part of the nitrifiable soil protein (8).

A high C : N ratio in soil would indicate that some conditions exist that prevent the micro-organisms from decomposing the carbonaceous residues efficiently. It has been suggested that heavily leached soils owe their high content of organic matter chiefly to climatic conditions in which the decomposition of plant residues proceeds slowly, and that leaching of the acidic products induces a continuation of low base-saturation after the soils have come into cultivation. The studies so far made of cultivated podsol soils of the Appalachian uplands of Quebec Province have shown that nitrogen alone, in the form of sodium nitrate, does not increase microbial activity. They have shown, also, that the addition of caustic amendments results in greater biological activity, not only in the total flora, but also in the formation of nitrates, thus indicating that nitrifiable material had been released from the organic complex; the freed nitrogen evidently enabled the heterotrophic organisms to decompose more of the carbonaceous residues in the soil (5).

Although soil organisms are able to decompose more cellulose in the presence of added nitrogen compounds than in their absence, considerable time is needed, and it is possible that some of the decomposition is due to other forms of nitrogen already present or developing in the soil. It is also probable that the decomposition is carried out by an association of organisms, and not by a restricted flora, the decomposition resulting in a number of products of different energy values; this may also be true in the decomposition of starch. It was thought, therefore, that it would be useful to study the course of the decomposition of substances that would encourage the rapid development of a restricted flora, in order to determine if the addition of nitrogen would stimulate the organisms during the utilization of material supplying readily available energy.

Under cultural conditions micro-organisms may produce glucose from cellulose (9), and maltose, with or without glucose, from starch. In view of the fact that glucose can be so easily decomposed by many micro-organisms, both aerobic and anaerobic, it seemed useful to investigate some of the factors that operate in the aerobic decomposition of glucose in soil samples under controlled conditions. The present study was made as an attempt to ascertain the effects of mineral and organic nitrogen compounds on the decomposition of glucose in samples of field soil of the type mentioned.

Experimental

Glucose is used in laboratory studies of microbial activities in soil, especially for obtaining enrichment cultures of nitrogen-fixing bacteria. When added

to soil under the appropriate conditions of oxygen tension, moisture and temperature, *Azotobacter* may develop in the presence of atmospheric oxygen, and *B. amylobacter* if the oxygen be reduced or removed.   It had been previously ascertained that *Azotobacter* was absent from these soils, even after the addition of lime.   The conditions of the experiments to be described were such that adequate aeration of the treated samples would have enabled an aerobic flora to develop, so that *B. amylobacter* or other anaerobic bacteria would not be encouraged.   It was, otherwise, no part of the present studies to ascertain the cultural characters of the bacteria that utilized the glucose.

The soil samples were taken from field plots under experiment in or near Sawyerville, Compton County, in the Eastern Townships district of Quebec Province.   The soils are derived from black and grey Ordovician slates, and have an organic carbon content of from 3 to 6%; the ratio of organic carbon to nitrogen is about 15 : 1 (4).   Samples were collected by spade from the top 6 in., passed through a 2-mm. sieve, and allowed to dry rapidly in the air of the laboratory.   After the glucose and nitrogen compounds had been added, the dry soils were moistened with distilled water to give 60% of the water-holding capacity, which was determined by the funnel method (10). The required amount of water was added slowly from a pipette; when the soil at the bottom of the dish was wet, the moist soil was thoroughly mixed.

The utilization of glucose, added as a 0.5% solution, was determined by the method of Bertrand (3).   Biological activity was determined by estimation of the amount of carbon dioxide evolved during a period of about 10 days at room temperature (25 to 28° C.).   The numbers of bacteria were estimated by plating with soil extract glucose agar, the same medium being used, after acidification with sterile tartaric acid solution, to count the fungi.   Nitrates were determined by Harper's modified phenoldisulphonic acid method (7).

For the evolution of carbon dioxide the treated samples were placed in suction flasks of 1000-ml. capacity, and the gas was absorbed by means of barium hydroxide, as described previously (10).   Samples for the determination of sugar, for numbers of bacteria and fungi, and for nitrates, were placed in large culture dishes, to permit adequate penetration of atmospheric oxygen.

The following experiments were made:

   (i) A preliminary experiment to ascertain the rate of decomposition of glucose in the presence and absence of nitrate nitrogen.

  (ii) An experiment to determine the effects of nitrate in single and double amounts.

 (iii) An experiment to determine the effects of soil nitrates (developed in samples incubated for 30 and .56 days) and the effects of a previous treatment with glucose.

 (iv) An experiment to determine the effects of the amino-acid glycine.

In each experiment a sample of soil having only water added to it was used as a control.

EXPERIMENT 1

The samples used in this experiment had been dried, before nitrate nitrogen could accumulate, and stored in the laboratory. A test showed that there was no nitrate nitrogen present in the soil. The treatments were (i) glucose alone, (ii) glucose plus potassium nitrate, and (iii) untreated control. The amount of potassium nitrate added was sufficient to give 16.7 p.p.m. of nitrate nitrogen in the moistened soil. The amount of glucose added was equivalent to 30.9 mg. per 10 gm. of moist soil.

The results of the determinations of glucose, of nitrate nitrogen, and of the estimations of bacterial numbers are given in Table I.

TABLE I

Time, hr.	Glucose in 10 gm. moist soil, mg.		Nitrate nitrogen, p.p.m.	Bacteria, millions per gm.		
	Glucose	Glucose +KNO₃	Glucose +KNO₃	Glucose	Glucose +KNO₃	Control
At start	29.0	28.0	16.9	0.9	0.9	0.9
12	28.0	28.0	13.8	1.0	1.0	1.1
24	27.0	26.0	15.9	2.3	3.1	6.5
48	15.4	9.5	Nil	31.1	40.1	15.8
72	4.4	1.1	—	35.2	33.8	18.2
96	Nil	1.1	—	42.0	40.9	15.6
120	Nil	Nil	...	40.7	42.0	23.5

The disappearance of the added nitrate, between the 24th and 48th hr., was associated with the sudden decrease in the amount of glucose and with the rise in bacterial numbers. The nitrate does not appear to have effected any difference in numbers; the difference observed between the amounts of glucose remaining at the 48th hr. may not be significant. The maintenance of high numbers after the sugar had been utilized is commonly found after treatments that stimulate a restricted flora.

The carbon dioxide production was determined from duplicate 100-gm. portions of soil; the total amounts of gas evolved from the cultures in 12 days are given below.

TABLE II

EVOLUTION OF CARBON DIOXIDE, MG. PER 100 GM. OF SOIL

Sub-sample	Glucose	Glucose +KNO₃	Control
a	571	584	260
b	553	572	254
Mean	562	578	257
Minus control	305	321	—

The nitrate appears to have increased activity. The effect can be seen by determining the percentage difference between the mean of the two cultures receiving glucose, after subtracting the mean values of the controls. The mean difference due to nitrate was only 5%, which, in view of the differences found between parallel sub-samples, cannot be considered significant.

Since, however, the effects of the nitrate may have occurred only during the time when the glucose was being utilized and while the bacteria were increasing in numbers, it seemed probable that the values for the amounts of carbon dioxide produced during that time would show greater differences. The amounts of carbon dioxide were therefore determined for each interval in the manner described below.

It was the practice in this and later experiments to titrate the barium hydroxide in the first two absorption tubes at intervals as seemed necessary, before all of the barium hydroxide had been changed to the carbonate; that in the third tube was titrated at the end of the experiment. In order to show the rate of evolution, values were derived by determining the proportion that the amounts found in the first tube at each interval bore to the total amount and by calculating the amounts in the second and third tubes as proportionate for each interval. The values given in Table III represent the calculated amounts in tubes 2 and 3 added to the known amounts in tube 1. The values may be accepted as true if it may be assumed that the amounts of carbon dioxide passing over from tube 1 to tube 2, and from tube 2 to tube 3, were regularly proportionate throughout the experiment. From the observed values, the amounts of carbon dioxide evolved per hour were calculated; these values are also shown in Table III.

The latter values bring out the fact that the increase due to nitrate was considerably higher during the earlier stages, namely, between the 32nd and the 50th hr. The period of greater effect corresponds to that during which the bacteria were increasing at the greatest rate, and that during which, as shown in Table I, the glucose was most rapidly decomposed.

TABLE III

EVOLUTION OF CARBON DIOXIDE FROM 100-GM. SAMPLES OF SOIL

Time, hr.	Total amount, mg.			Rate, mg. per hr.		
	Glucose	Glucose +KNO₃	Control	Glucose	Glucose +KNO₃	Control
25.50	61.85	54.37	40.42	2.43	2.13	1.59
32.25	48.65	52.45	–	7.21	7.77	–
41.75	54.32	76.06	28.45	5.73	8.02	1.75
49.25	37.58	47.08	–	5.01	6.28	–
65.75	63.40	66.35	–	3.84	4.02	–
80.25	47.74	41.37	45.70	3.29	2.85	1.19
120.00	75.64	74.25	30.53	1.89	1.86	0.76
168.00	66.06	64.04	38.25	1.38	1.34	0.80
288.00	107.04	102.33	73.54	0.89	0.84	0.60

EXPERIMENT 2

Since 16.7 p.p.m. of nitrate nitrogen stimulated the evolution of carbon dioxide, an experiment was made to test the effect of double that treatment. In this experiment, four 600-gm. samples of fresh air-dried soil containing no nitrate nitrogen were treated as follows: (i) 3.0 gm. glucose; (ii) 3.0 gm. glucose, 0.195 gm. potassium nitrate; (iii) 3.0 gm. glucose, 0.390 gm. potassium nitrate; (iv) no treatment. A 100-gm. portion of the moist soil of each treatment was used for determination of carbon dioxide production; the remainder was incubated in the dish for determination of glucose and nitrate, and the numbers of bacteria and fungi.

The amounts of glucose found in the samples are shown in Table IV.

TABLE IV

GLUCOSE, MG. PER 10 GM. OF SOIL

	Glucose	Glucose +KNO₃	Glucose +KNO₃ × 2
Glucose added	30.8	30.8	30.8
Found, at start	28.4	28.4	28.4
Day 1	19.0	20.3	19.6
Day 2	6.0	5.0	7.5
Day 3	Nil	Nil	Nil

The glucose disappeared at the same rate in the three cultures.

The amounts of carbon dioxide collected in 276 hr. were as shown in Table V.

TABLE V

EVOLUTION OF CARBON DIOXIDE FROM TREATED SOIL

	Glucose	Glucose + KNO₃	Glucose + KNO₃ × 2	Control
	Milligrams per 100 gm. of soil			
From cultures	373	405	406	95
Minus control	278	310	311	—
Time, hr.	Milligrams per 100 gm. per hr.			
22.0	3.12	2.70	2.49	—
36.0	4.94	6.04	5.14	—
51.0	3.90	4.60	5.23	—
75.5	2.32	2.30	3.10	0.52
123.0	0.82	0.95	1.02	—
192.0	0.69	0.75	0.70	0.31
276.0	0.42	0.52	0.32	0.23

The effect of nitrate nitrogen, estimated after subtracting the value of the control culture, was to increase the total carbon dioxide by 11.5%.

The amounts of carbon dioxide collected at intervals were also calculated, in the manner described, in order to arrive at the effects of the treatments on the rates of evolution; the results are given in Table V.   The nitrate appears to have depressed the evolution of carbon dioxide at the start, but to have increased it after the 24th hr.   The double amount was the more effective between the 3rd and 5th days.

The amounts of nitrate found in the cultures to which it was added, and the numbers of bacteria in all cultures, are given in Table VI.   The nitrate was apparently being utilized at about the same rate in both cultures to which it was added, until the end of the 3rd day, after which 53 to 55% of the nitrate in the culture with the double amount remained until the end of the experiment.

TABLE VI

Time, days	Glucose	Glucose + KNO_3	Glucose + $KNO_3 \times 2$	Control
	Nitrate nitrogen, p.p.m.			
At start	—	33.4	60.0	Trace
1	—	—	—	Trace
2	—	13.2	47.0	Trace
3	—	Trace	27.1	—
5	—	—	27.1	—
12	Nil	Nil	28.5	18.9
	Bacteria, millions per gm.			
At start	5.2	—	—	—
1	63.9*	44.8	36.8	10.6*
2	154.5	203.0*	252.8	4.9
3	97.5	192.0	151.6*	5.6
4	120.1	145.0	135.2	6.8
5	46.9	46.1	32.8	7.6
	Fungi, thousands per gm.			
7	143	196	220	118

* χ^2 *excessive.*

The increases in the amounts of carbon dioxide, in the cultures receiving nitrate, may be ascribed to the higher numbers of bacteria that developed during the period synchronizing with the greater rate of evolution of the gas. The nitrate appears also to have depressed the increase in bacterial numbers at the 24th hr.   (See also Fig 1.)

The numbers of fungi were estimated on the 7th day in plates of soil extract glucose agar rendered acid with tartaric acid.   While not conclusive evidence of the course of development of these organisms, the numbers found are of interest, and are given in Table VI.
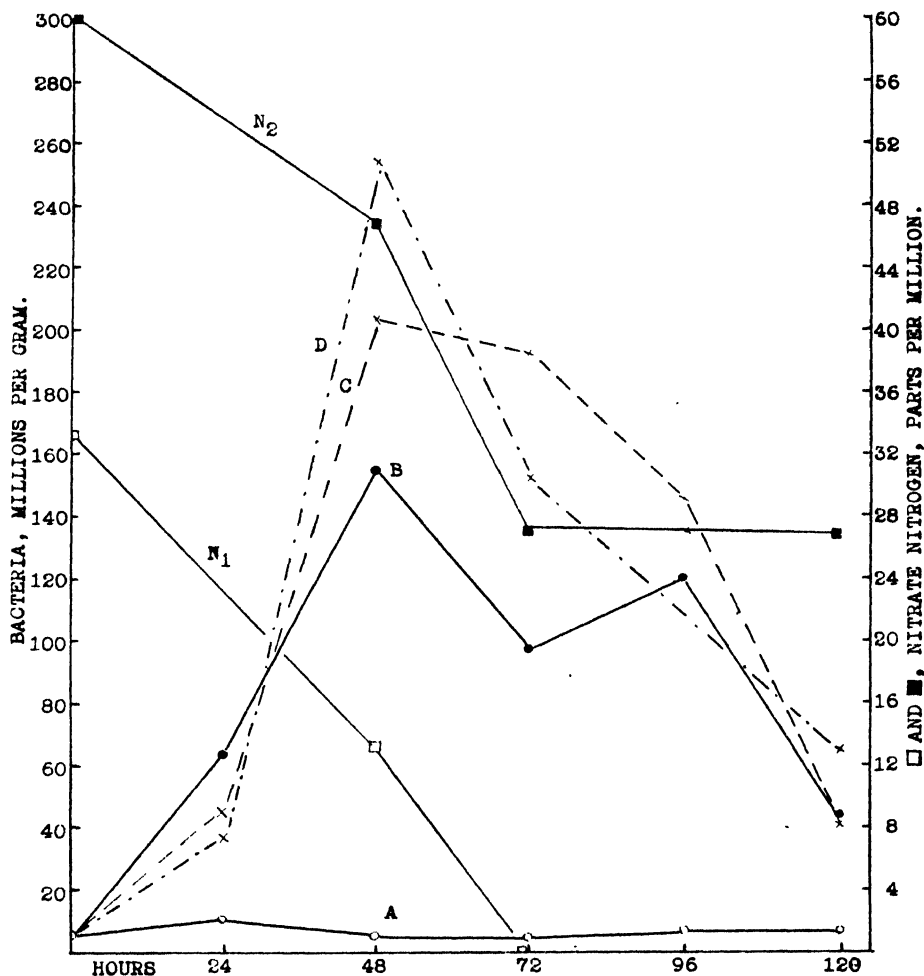
FIG. 1. Numbers of bacteria in:— A, control; B, glucose; C, glucose + potassium nitrate; D, glucose + potassium nitrate (double amount); and nitrate nitrogen in C (N_1) and D (N_2).

As shown in a later experiment, the fungi developed slowly, and reached their maximum numbers after the glucose had been utilized, during the period that bacterial numbers were decreasing.   This would suggest that some source of organic carbon, and with it some organic nitrogen, or ammonia, were released during that period.

It would appear that the complete utilization of the sugar at about the end of the 3rd day set a limit to the further use of about half of the nitrate in the sample receiving the double amount.   The fact that bacterial numbers fell rapidly with the disappearance of the glucose and remained stationary between the 5th and the 11th days suggested that there was no further source of energy remaining for these aerobic bacteria; that is, the glucose was completely

oxidized. The carbon dioxide found, therefore, after the 3rd day must have been due to respiration of diminished numbers of bacteria, coupled with that of apparently increasing numbers of fungi, developing from some source of energy released through bacterial action and from a source of nitrogen other than nitrate nitrogen.

The maximum rate of evolution of carbon dioxide occurred at about the 36th hr., which corresponds to the time when half of the energy material had been utilized (Table V). In view of the fact that the rate of evolution at the 75th hr. was, on the average, about half the rate at the 36th hr., it is probable that the whole of the glucose was utilized at about the 72nd hr., when the final determination was made for glucose. This seems to be a reasonable assumption, as half the glucose had gone at the 36th hr. About half of the nitrate nitrogen in the culture that received the single amount had also been utilized at that time.

It may be useful to submit a calculation regarding the relation of carbon dioxide produced to the theoretical amount possible at the 72nd hr. The amount of gas evolved from the sugar at that time may be taken as 220 mg., which represents about 41% of the theoretical maximum amount. The total amount of gas produced from glucose in the culture with added glucose only was 373 − 95 mg. = 278 mg.; this represents 66.7% of the theoretical maximum amount. The average amount evolved in 11.5 days from the three cultures receiving glucose, 300 mg., represents 72%. Merrill (11) states that in pure cultures of *Mycobacterium*, a group of aerobic bacteria that oxidize glucose, 85% of the theoretical maximum amount may be obtained. In soil cultures there is the probability to be considered that products other than carbon dioxide may be formed and utilized.

If the rates of evolution at 276 hr. be compared, it is clear that some time would elapse before the rates from the cultures with glucose would fall to the value given by the control soil.

Tests by the colorimetric method with samples that had an initial pH of 5.06 showed that after glucose had been decomposed the pH had been raised to 7.0; distillates from the samples were also approximately neutral.

It is clear that there was sufficient nitrogen available in the soil to allow the bacteria in the sample receiving glucose alone to utilize all of the available sugar in the same time as in the samples receiving nitrate.

EXPERIMENT 3

The samples of soil used in the previous experiments contained no nitrate nitrogen other than that supplied as potassium nitrate. In this experiment, samples of fresh soil from the same source were incubated for a sufficient length of time to allow the nitrifying bacteria to develop a considerable content of nitrate through the oxidation of the ammonium, or amino-, compounds of the soil protein. The effect of the removal, or at least of the utilization, of available nitrogen by previous decomposition of glucose was also tested.

After the soils had been incubated in the moist state, for different lengths of time, they were spread out to dry rapidly. The treatments are summarized in Table VII. Nitrates were then determined, and plates prepared for counting bacteria and fungi; glucose was then added and the samples were moistened. It was expected that some of the effects might be due to the length of time the samples had remained in the air-dry state; therefore, a sample of each series without glucose was also moistened and incubated, to serve as a control. The results are given in Tables VII, VIII, and IX.

As shown in Table VII, the glucose disappeared rapidly, all of it being utilized within 48 hr. The nitrate nitrogen does not appear to have stimulated the decomposition.

TABLE VII

TREATMENT OF SAMPLES, NITRATE CONTENT, AND UTILIZATION OF GLUCOSE

Series	Incubation previous to air-drying and moistening, days	Time air-dried, days	Nitrate N after air-drying, p.p.m.	Glucose added per 10 gm. soil, mg.	Glucose found, mg.		
					At start	Day 1	Day 2
I	Nil	71	Trace	30.8	30.5	17.9	Nil
II	30	41	32–33	30.8	30.5	18.2	Nil
III	56	15	41–42	30.8	30.5	19.4	Nil
IV	30 with glucose	41	Trace	30.8	30.5	12.0	Nil

The evolution of carbon dioxide in 10 days and the amounts produced per hour are shown in Table VIII.

TABLE VIII

EVOLUTION OF CARBON DIOXIDE FROM 100-GM. SAMPLES OF SOIL

Series		I	II	III	IV
		Total amount in 10 days, mg.			
Cultures with glucose		418	404	424	468
Control cultures		120	94	77	178
Minus controls		298	310	347	290
	Time, hr.	Rate, mg. per hr. (from first 2 baryta tubes)			
Cultures with glucose	24	3.20	3.22	2.62	4.07
	36	7.05	7.59	8.89	7.75
	55	4.56	4.92	5.78	4.48
	144	0.86	0.85	0.86	0.86
Control	55	0.93	0.72	0.61	1.37

In a discussion of the results, it is first necessary to consider the effects of drying for the different periods. The effect can be seen best in the control cultures of Series I, II, and III, which had remained in the air-dry state for 71, 41, and 15 days respectively. The longer the period of drying, the greater was the amount of gas evolved. The effects were masked by the decomposition of glucose to such an extent that, in considering the total amount of gas produced from the cultures, no real differences can be seen. The amounts of carbon dioxide derived from the glucose may, therefore, be ascribed to other factors affecting the micro-flora, which by the treatment with glucose became restricted to one or two types of micro-organisms. The amount of gas produced from glucose in Series II, which contained 32–33 p.p.m. of nitrate nitrogen, was about 4% more than that from Series I; that from Series III, about 16%. In view of the close agreement obtained between duplicate cultures in a later experiment, reported below, it is possible that both of those differences are significant.

The effects of the previous treatment with glucose can be seen both in the control cultures and in the cultures receiving the second addition of glucose; the comparison should be made only between the cultures of Series II and IV, which had remained dry for the same number of days. Previous decomposition of glucose did not increase the amount of carbon dioxide produced from the second addition, but reduced it by about 7%, which may be significant. The previous treatment did, however, stimulate a more rapid initial evolution after the second addition, the increased rate, calculated for the 24th hr., being 57% higher. The greater effect of the previous treatment is seen in the control cultures, in which the increase in the total amount of gas was 89%.

It would appear, then, that the previous development of organisms set up some condition that did not stimulate the utilization of another supply of the same energy material, except in the first few hours. The decomposition of glucose in this soil seems, therefore, to be of a nature different from that taking

TABLE IX

NUMBERS OF BACTERIA AND FUNGI, INCUBATION EXPERIMENT

Series		I	II	III	IV	I	II	III	IV
	Time, days	Bacteria, millions per gm.				Fungi, thousands per gm.			
Cultures with glucose	At start	1.3	2.4	3.3	8.4	98	74	90	132
	1	19.7	19.8	8.1	25.5	28	7	53	53
	2	29.3	47.1	57.1	62.1	62	100	72	155
	3	63.3	59.3	53.6	87.1	82	217	290	286
	4	37.4	73.5	86.2	76.0	110	342	457	412
	6	47.3	61.1	69.4	78.4	162	507	610	592
Control cultures	At start	1.3	2.4	3.3	8.4	98	74	90	132
	2	4.4	5.6	5.1	2.2	90	104	86	119
	4	14.4	9.3	9.7	34.7	170	115	147	167

place when successive additions of a hydrocarbon are decomposed. Sen Gupta (13) showed that second and third additions of phenol were decomposed at increasingly rapid rates in Rothamsted soil.

The numbers of bacteria and fungi are shown in Table IX. From the results shown for bacterial numbers, it would appear that the nitrate formed in the incubated soil had no influence on the development of these organisms. The increased numbers of fungi in Series II and III suggest that they were stimulated by the nitrates, but the numbers in Series IV would rule out that suggestion. The increases in the numbers of the fungi would seem to confirm the view expressed above, namely, that bacterial activity may have released some form of food material, containing both carbon and nitrogen, for these organisms. The higher numbers in the control culture of Series IV at the start gives emphasis to this view.

EXPERIMENT 4

This experiment was made to ascertain if an amino-acid would serve as a source of carbon and nitrogen, and if it would stimulate the decomposition of glucose.

The samples were derived from the same source as those used in the two previous experiments. There was only a trace of nitrate nitrogen present.

The following cultures were made: (i) 400 gm. of soil, with 2.0 gm. of glucose; (ii) 400 gm. of soil, with 0.112 gm. of glycine; (iii) 400 gm. of soil, with glucose and glycine; (iv) no treatment.

The glycine supplied 52 p.p.m. of nitrogen. Duplicate 100-gm. portions of soil were used for the evolution of carbon dioxide; the remainder was used for the determination of glucose, and for the estimation of numbers of bacteria and fungi. The results are shown in Tables X, XI, and XII.

The glucose was used up rapidly, none being left in the soil on the second day.

The amounts of carbon dioxide collected in 10 days were as shown in Table X.

TABLE X
CARBON DIOXIDE, MG. PER 100 GM. OF SOIL

	a	b
Soil + glucose	401	401
Soil + glycine	153	157
Soil + glucose + glycine	461	464
Control	113	113

The amino-acid evidently stimulated biological activity to an appreciable extent: the increase of the glycine cultures over the control cultures was 37%; the increase caused by glycine in the cultures receiving both chemicals over those receiving glucose only, was 15%. In view of the close agreement between duplicate cultures, this is probably a real difference. The differences among

the effects of the treatments are more clearly brought out by comparing the rates of evolution, given in Table XI.

TABLE XI

CARBON DIOXIDE, MG. PER 100 GM. PER HOUR

Time, hr.	Cultures receiving glucose			
	Glucose		Glucose + glycine	
	a	b	a	b
28.5	3.02	2.99	2.92	2.92
36.5	8.60	9.31	10.27	10.55
46.0	5.58	5.38	9.37	9.66
60.5	4.37	4.40	4.03	4.87
142.0	1.05	1.04	1.12	1.11
238.5	0.46	0.44	0.44	0.45
	Cultures without glucose			
	Glycine		Control	
	a	b	a	b
70	1.26	1.35	0.92	0.92
238.5	0.39	0.37	0.29	0.29

Although glycine alone stimulated activity to such an extent that the rate of production was increased 41% at the end of the 70th hr., the greater effect of the glycine occurred in association with glucose; at the 46th hr. the increase due to glycine was 77% (see also Fig. 2).
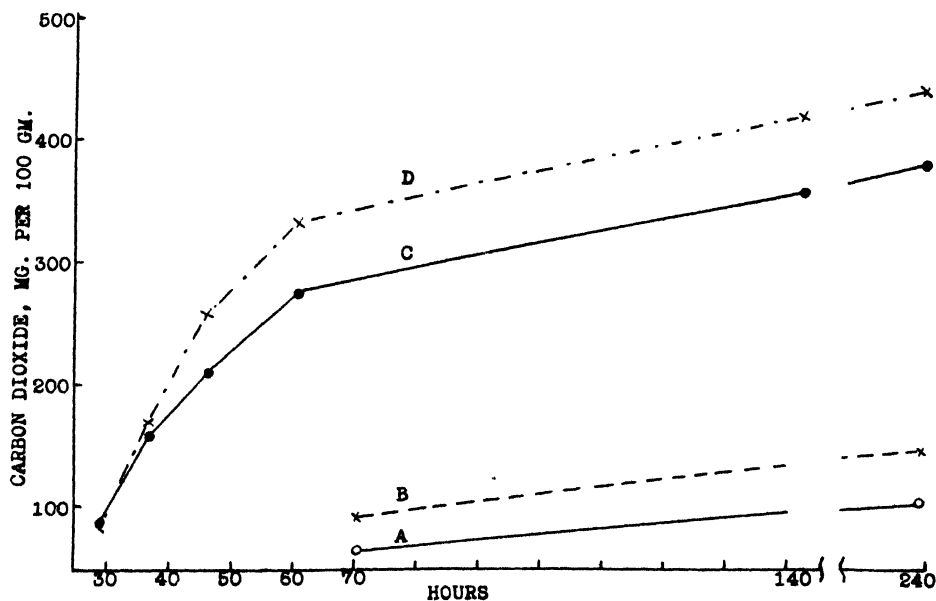
FIG. 2.   *Cumulative evolution of carbon dioxide from:— A, soil alone; B, soil + glycine; C, soil + glucose; and D, soil + glucose + glycine.*

TABLE XII

NUMBERS OF MICRO-ORGANISMS, GLYCINE EXPERIMENT

Time, days	Glucose	Glucose + glycine	Glycine	Control
	Bacteria, millions per gm.			
At start	1.9	1.9	1.9	1.9
1	16.2	4.3	2.7	5.8
2	84.7	45.4	5.0*	9.2
3	66.9	73.2	14.1	8.3
4	74.4	83.1	26.1	10.5
7	75.4	71.7	37.5	16.5
	Fungi, thousands per gm.			
At start	101	101	101	101
1	197	212	41	107
2	110	232	100*	86
3	282	800	220	112
4	287	1060	195	102
7	570	960	267	77

* Approximately; too many colonies in the plates for an accurate count.

Bacterial numbers were depressed by the glycine until after the 48th hr., when they began to rise and were nearly double the numbers in the control cultures at the 4th and 7th days.

Reference to Table XII shows also that the numbers of bacteria in the samples receiving glucose were not increased by the glycine, but were depressed until the 4th day. The increase in the carbon dioxide could not therefore be ascribed to the bacteria alone, since at the time of the greatest increase in rate, at the 46th hr., the numbers in the culture with added glucose and glycine were about equal to only half the numbers that developed in the sample with glucose alone. The fungi, on the other hand, were greatly stimulated by the glycine added with the glucose; the greater effect was not evident until the glucose had disappeared. The increase in numbers of fungi caused by the glycine was relatively less than that of the bacteria; by taking the mean numbers of the two cultures with glucose, it will be seen that the fungi multiplied from 7 to 8 times in 7 days, while the bacteria increased about 35 times in 3 days. Most of the carbon dioxide must have been produced by the bacteria, whose numbers did not become less while the fungi were increasing. Numbers alone, however, are not an adequate basis of comparison between these two kinds of micro-organisms, as they differ not only in size, but also in their mode of using sources of energy and nutrition.

The decomposition of glucose evidently released some source of food that enabled the fungi to develop to a considerable extent after the bacteria had multiplied; this was confirmed in another experiment. The glycine, in the presence of glucose, was apparently utilized by the fungi at the time that the

bacteria were also increasing in numbers, but the competition of the increasing fungi caused the bacteria to multiply at a slower rate than that produced by glucose alone.

The results of this experiment suggest that the natural flora had been displaced not only by a selective group of bacteria that obtain their energy from the added carbohydrate, which enables the cells to utilize some source of organic nitrogen, but also by a group of fungi that develop by utilizing decomposition products of the glucose or material released from the organic matter during and following the destruction of the carbohydrate. It would also appear that the fungi, developing somewhat more slowly than the bacteria, preferred nitrogen derived from the added amino-acid rather than the nitrogen in the soil. Reference to the results of the counts for fungi in Experiments 3 and 4 would seem to confirm this, and to suggest that nitrates, either added in the form of potassium nitrate or developed through nitrification, are less suitable sources of nitrogen than glycine for the fungi in soil.

Conclusions

The results from these experiments with samples of field soil would seem to confirm the view expressed in a previous paper dealing with virgin soils (6), namely, that water-soluble materials in soil include substances that are able to support the growth of micro-organisms.

In regard to the cultivated soils studied in the present work, the relation between the various factors in the complex material that supplies energy or food for the micro-flora is somewhat different from that in virgin soils of this type; the organic carbonaceous residues are in a more advanced stage of decomposition, as is shown by the higher levels of numbers of organisms and by the lower yield of carbon dioxide (6) in the cultivated soils. This would suggest a reason for the fact that nitrate nitrogen alone does not stimulate further development of the micro-flora. It would appear from the results reported above that the nitrogenous material remains in a state of availability, or quickly becomes available, because additional sources of easily available carbon are rapidly destroyed by an increasing number of bacteria. The development of filamentous fungi in the soils after the decomposition of the carbonaceous material, and after the bacterial numbers have reached their peak, suggests that food material containing both carbon and nitrogen had been released.

The experiments reported here can be regarded only as part of an attempt to determine the nitrogen metabolism of aerobic soil micro-organisms, which probably, under normal cultural practices, are responsible for the decomposition of soluble carbonaceous compounds in the organic residues in soil. The method adopted was designed to stimulate only those groups of organisms able to utilize a single source of additional energy, within a period of time in which it would be possible to evaluate their action without the interference of competitive groups, such as may develop when a more complex material,

for example cellulose, is added to soil. It seems clear that although nitrate nitrogen stimulated the evolution of carbon dioxide during the period of maximum development of bacteria, the additional nitrogen did not enable the organisms to utilize the glucose more rapidly than they did in soil to which nitrate nitrogen was not added.

Acknowledgment

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SOME *PEZICULA* SPECIES AND THEIR CONIDIAL STAGES[1]

By J. WALTON GROVES[2]

Abstract

The following species of *Pezicula* have been cultured and the genetic connection of their conidial stages has been established; *Pezicula carpinea* (Pers.) Tul. with the conidial stage *Cryptosporiopsis fasciculata* (Tode) Petr., *Pezicula pruinosa* Farl. with the conidial stage *Sphaeronema pruinosum* Peck, *Pezicula Corni* with the conidial stage *Cryptosporiopsis cornina* (Peck) Petr. and Syd., *Pezicula Rubi* (Lib.) Niessl with the conidial stage *Discosporiella phaeosora* (Sacc.) Petr., and *Pezicula Hamamelidis* n. sp. with the conidial stage a *Cryptosporiopsis* species. The form of the conidial spore is relatively constant in the different species, but the form of the conidial fruiting body is variable.

Introduction

It has been observed by mycologists from time to time that morphological correlations may exist between the perfect and imperfect stages of pleomorphic fungi, and, where such correlations do exist, they are of value in indicating relationships. The purpose of this paper is to describe, in some detail, certain species of the genus *Pezicula* and their conidial stages. The genetic connections have been established by cultural methods, and the conidial stages have proved to be of interest in that, in the different species, the form of the conidial spore remains relatively constant while the form of the fruiting body may be quite variable. The species to be discussed in this paper have been chosen for the purpose of illustrating the range of variation that has been found.

The genus *Pezicula* was erected by Tulasne (21), based on *Peziza carpinea* Pers., which he transferred from *Dermatea* Fries. In his account, Tulasne drew attention to the form of the conidia, which were stated to be ovate to oblong; and he described three other species, *Pezicula Coryli* Tul., *P. amoena* Tul., and *P. dissepta* Tul., which were also transferred from *Dermatea* where he had referred them in an earlier paper (20). The close relationship of *Dermatea* and *Pezicula* has been generally recognized by later authors, some of whom have not recognized *Pezicula* as distinct, but no attempt will be made here to trace the various generic concepts.

Pezicula species are typically bright coloured, waxy-fleshy in consistency, with rather broad asci, oblong-ellipsoid ascospores, and oblong-ellipsoid

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conidia; while *Dermatea* species are typically dark coloured, more leathery in consistency, usually with relatively narrower asci, more fusiform-elliptical ascospores, and elongate to sub-filiform conidia. It has been pointed out by Groves (6) that exceptional species such as *Dermatea acerina* (Peck) Rehm occur, in which oblong-ellipsoid conidia are found with a *Dermatea* type of apothecium, but such species only serve to emphasize the close relationship between the two genera. The fact that the majority of *Pezicula* species do differ from the majority of *Dermatea* species in both the perfect and imperfect stages would seem to be sufficient justification for continuing to recognize *Pezicula* as a distinct genus.

The material on which this study is based was collected, for the most part, in the Temagami Forest Reserve, Ontario, and in the vicinity of Toronto, Ontario. Cultures were obtained from both ascospores and conidia and were grown on 2% malt extract agar and on sterilized twigs of the host. The twig cultures were prepared as described in an earlier paper, Groves (5). In only one of these species was the perfect stage obtained in culture, but the fact that the cultures from ascospores and conidia were similar and that both produced the same type of conidial fruiting body and spores in culture, was considered sufficient evidence of genetic connection.

Description of Species

Pezicula carpinea (Pers.) Tul.   Sel. Fung. Carp. 3 : 183.   1865.

> *Peziza carpinea* Pers.   Syn. Fung. p. 673. · 1801.
> *Cycledum carpinea* Wallr.   Flor. Crypt. Ger. 2 : 512.   1833.
> *Dermatea carpinea* Fries.   Summa veg. Scand. p. 362.   1849.
> *Octospora paradoxa* Hedw.   Obs. Bot. 1 : 13.   1802.
> *Ditiola paradoxa* Fries.   Syst. Myc. 2 : 171.   1822.
> *Ombrophila paradoxa* Sacc.   Syll. Fung. 8 : 620.   1889.
> *Pezicula fasciculata* Dearn. and House.   N.Y. St. Mus. Bull. 243 : 244. 1921.
> *Tubercularia fasciculata* Tode.   Fung. Meckl. tab. IV, fig. 3.   1790.
> *Cryptosporiopsis fasciculata* Petrak.   Ann. Myc. 21 : 186.   1923.
> *Discella discoidea* Cke. and Pk.   Ann. Rep. N.Y. St. Mus. 28 : 58. 1876.
> *Discula Peckiana* Sacc.   Syll. Fung. 3 : 675.   1884.
> *Peziza Betuli* Alb. and Schw.   Consp. Fung. p. 309.   1805.
> *Stictis Betuli* Fries.   Syst. Myc. 2 : 195.   1822.
> *Ocellaria Betuli* Rehm.   Rab. Kr. Fl. I, 3 : 136.   1889.
> *Tuberculariella Betuli* v. Höhn.   Z. Gärungsphysiol. 5 : 209.   1915.
> *?Dermatella scotinus* Morg.   J. Myc. 10 : 98.   1904.

Apothecia (Fig. 1 : 1) strongly erumpent, more or less in rows or sometimes scattered, caespitose, usually in elongated clusters, occasionally single, circular or distorted by crowding, narrowed below, substipitate, 0.5–1.0 mm. in diameter, 0.5–1.0 mm. in height, pale ochraceous yellow, slightly pruinose, brittle, rather waxy in consistency, more fleshy when moist; hymenium plane

to convex, slightly pruinose, ochraceous yellow to reddish orange, much brighter when moist, at first with a delicate lighter border which may disappear later; tissue of the hypothecium pseudoparenchymatous at the base, composed of irregular, hyaline cells, about 5–12 μ in diameter, often with host cells intermingled, becoming more prosenchymatous above, composed of more or less vertically parallel hyphae, 3–5 μ in diameter, in the upper part more interwoven and looser, often with intercellular spaces,
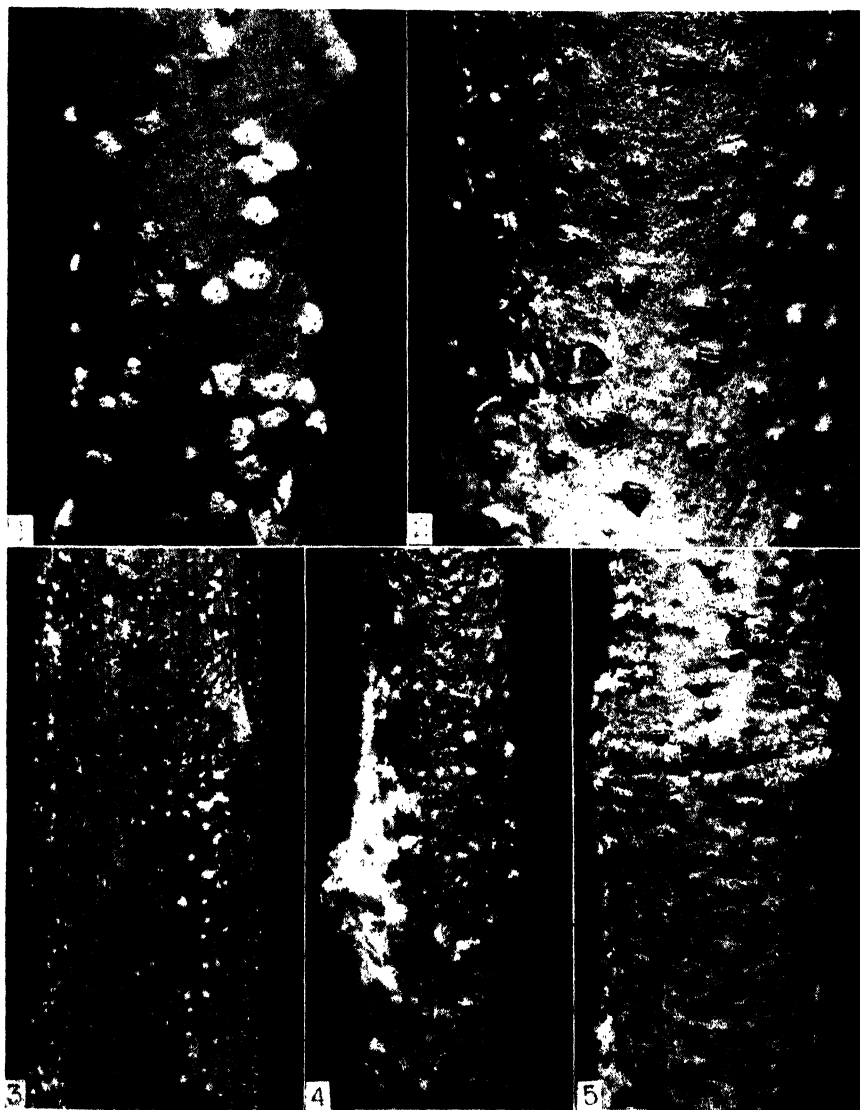
FIG. 1. 1. Apothecia of Pezicula carpinea; 2. Conidial stage of P. carpinea; 3. Conidial stage of P. Rubi; 4. Apothecia of P. Rubi developed in culture; 5. Apothecia of P. Corni. All ×4 approx.

toward the outside forming a pseudoparenchymatous zone, a few cells in thickness, of irregular, more or less isodiametric cells; subhymenium a narrow zone of slender, loosely interwoven hyphae; asci cylindric-clavate, usually tapering into a long stalk, eight-spored, sometimes four-spored, (120)–130–160–(190) × (14)–17–20–(22) μ; ascospores oblong-ellipsoid to ovoid, hyaline, straight or slightly curved, one- to four-celled, obliquely uniseriate or overlapping, (17)–20–30–(33) × (8)–10–12–(14) μ; paraphyses hyaline, filiform,
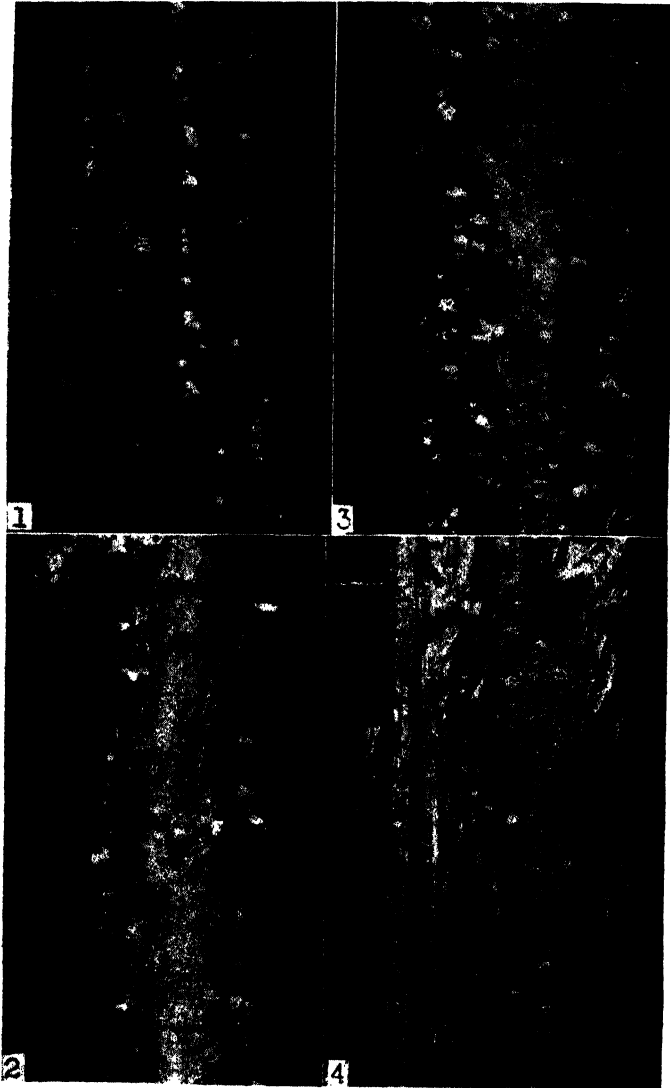
FIG. 2.   *1. Apothecia of* Pezicula *pruinosa; 2. Conidial stage of* P. pruinosa; *3. Conidial stage of* P. Corni *developed in culture; 4. Apothecia of* P. Rubi.   *All* ×4 *approx.*

septate, simple or branched, 1.5–2.5 μ in diameter, the tips swollen, up to 6 μ in diameter and forming a slight epithecium. (Fig. 4 : 1b.)

Conidial fruiting bodies (Fig. 1 : 2) erumpent, more or less in rows or scattered over the twigs, cushion-shaped or wart-like, almost circular or elongated, 1–3 mm. in diameter, 0.3–1.0 mm. in height, dark reddish-brown, greyish, olivaceous, or almost black, hard, waxy, more fleshy when moist; tissue com-

FIG. 3.   *1. Apothecia of* Pezicula Hamamelidis; *2. Conidial stage of* P. carpinea *developed in culture; 3. Conidial stage of* P. pruinosa *developed in culture; 4. Conidial stage of* P. Rubi *developed in culture; 5. Conidial stage of* P. Hamamelidis *developed in culture.   All* × *4 approx.*

posed of hyaline or pale yellowish, much branched, closely interwoven hyphae about 3–5 μ in diameter, in the upper central part more or less vertically parallel, spreading out obliquely toward the outside; conidiophores hyaline, septate, simple or branched, very variable in length, up to 100 μ, 2.5–3.0 μ in diameter and swollen up to 5–7 μ just below the tip; conidia oblong-ellipsoid, hyaline, straight or slightly curved, one end with a truncate apiculus, one-celled, 20–30–(34) $\times$ (10)–12–15 μ; microconidia hyaline, filiform, straight or curved, one-celled, 8–15 $\times$ 1.5–2.0 μ.   (Fig. 4 : 1a.)
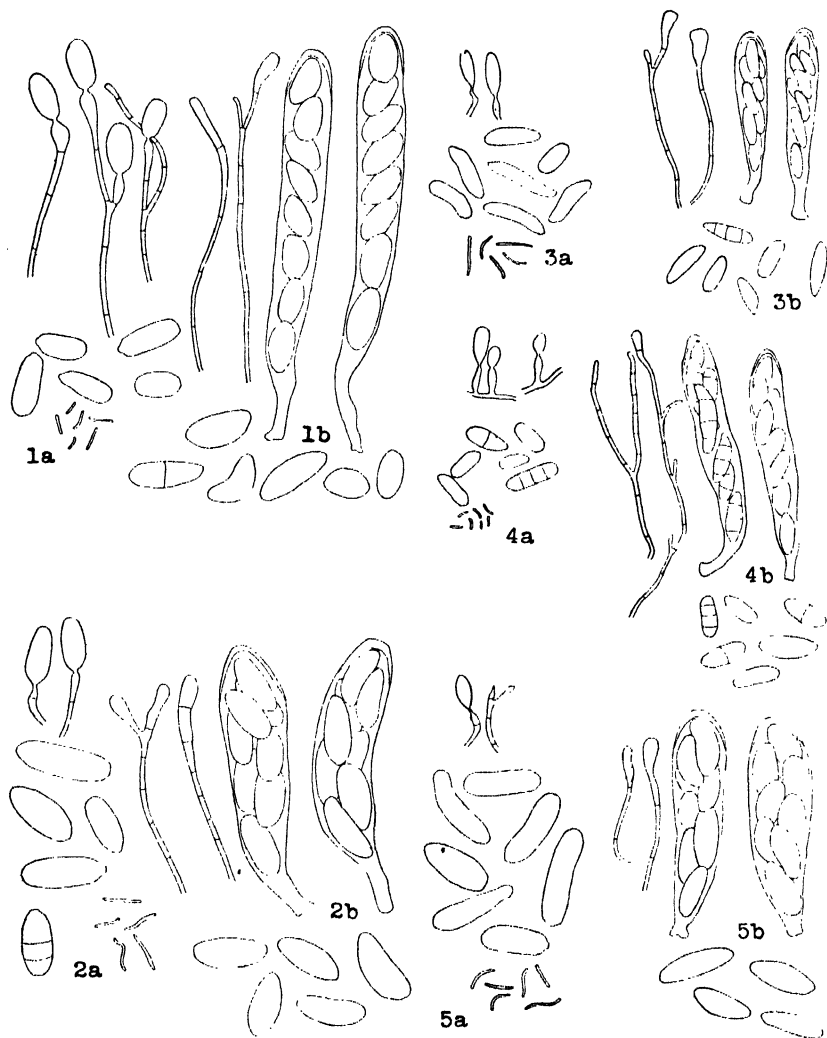
FIG. 4.   *1.* Pezicula carpinea. *a. Conidia, conidiophores, and microconidia.   b. Asci, ascospores, and paraphyses; 2.* P. Corni. *a. Conidia. conidiophores, and microconidia.   b. Asci, ascospores, and paraphyses; 3.* P. Rubi. *a. Conidia. conidiophores, and microconidia. b. Asci, ascospores, and paraphyses; 4.* P. pruinosa. *a. Conidia, conidiophores, and microconidia. b. Asci, ascospores, and paraphyses; 5.* P. Hamamelidis. *a. Conidia, conidiophores, and microconidia. b. Asci, ascospores, and paraphyses.   All* $\times 400$.

HOST: *Carpinus caroliniana* Walt.

EXSICCATI: Ell. N. Am. Fung. *67b* (as *Dermatea carnea*), *2623, 2741, 3333;* Shear N. Y. Fung. *93;* Rel. Farl. *134;* Fung. Col. *921;* Wils. and Seav. Asc. and L.F. *91;* Krieg. Fung. Sax. *1029;* Weese Eum. sel. exs. *450, 452;* Syd. Myc. Germ. *2729.*

SPECIMENS EXAMINED: University of Toronto Herbarium. On *Carpinus caroliniana* Walt. *2905, 3949 (65)*, 4377 (64), 7268 (287), 7394 (160),* Toronto, Ontario—*6019 (157),* Lynden, Ontario—*6570,* Brant Co., Ontario —ex U.S.D.A. Herb. F. P. *63042,* North Bloomfield, Conn.—ex Herb. Univ. Wis., Nekoosa, Wis., coll. J. J. Davis, June 8, 1918.

On *Carpinus* sp. Ex. Herb. Barbey-Boissier, Rel. Herb. Fuckel, Fung. rhen. *1129.*

Herbarium of J. W. Groves. On *Carpinus caroliniana* Walt. *58, 59,* Toronto, Ontario—*570,* Constance Bay, Ontario—*264* Ithaca, N.Y.

On malt extract agar the colonies reach a diameter of about 3 cm. in three weeks. They are white, or cream coloured to buff, or slightly yellowish, with abundant, fluffy, aerial mycelium which is more or less tufted or in cushion-like heaps. They develop circular to elongated, rounded, fleshy stromata, up to 3 mm. in diameter and 2 mm. in height, which usually very soon become covered with white, downy mycelium and remain sterile, but occasionally produce conidia. In the few instances in which the production of conidia was observed, the conidiophores appeared to be enclosed at first in a more or less circular cavity in the stroma. This cavity soon split open widely at the top and the fruiting surface became convex and wholly exposed. The microscopic structure was similar to that found in nature, but the tissue is sometimes looser, especially at the outside. Both macro- and microconidia were produced. Sporulation seems to be rare, and when it does occur lasts only for a brief period, as the stroma quickly becomes overgrown with vegetative hyphae.

On twigs of *Carpinus*, aerial mycelium is usually very abundant, white, cream coloured, or buff, forming cottony cushions on the twigs or sometimes completely covering them. Sporulation is rare on the twigs also. The fruiting bodies (Fig. 3 : 2) are erumpent, fleshy, rounded, or wart-like as in nature, 1 to 2 mm. in diameter and about 0.5 mm. in height, at first whitish, becoming reddish-brown and harder on drying. The conidiophores are borne over the outer surface and have not been observed to arise in a cavity. The microscopic features are similar to those found in nature. These fruiting bodies are also shortlived, and in a few days appear only as tufts of vegetative hyphae. It has been observed that if the conidial fruiting bodies as found in nature are put in a moist chamber, they very soon develop vegetatively in a similar manner.

Pezicula carpinea, as noted above, is considered to be the type species of the genus *Pezicula*. Its chief distinguishing characters are its occurrence on *Carpinus*, the yellow, waxy-fleshy apothecia in strongly erumpent clusters,

* The numbers in brackets refer to duplicate collections in the writer's herbarium.

the long asci, and the *Tubercularia*-like conidial stage. Brefeld (3) studied this species in culture, and his description of the cultures agrees well with the writer's cultures. He failed to obtain any fruiting bodies in culture, but this is not surprising as they are evidently formed only rarely, and then may easily be overlooked as the fruiting period is very brief.

This species is undoubtedly parasitic. Wagner (22) reported successful inoculations, and stated that in a 70- to 80-year-old stand of *Carpinus*, 140 out of 200 trees were killed by this fungus over a period of 10 years. On May 15, 1933, the writer inoculated two branches of a tree of *Carpinus caroliniana* in the following manner. A slit was cut in the bark with a flamed scalpel, and some agar and mycelium from a polysporous ascospore culture were inserted into the slit. The slit was then covered by a celluloid cylinder with the ends plugged with moist sphagnum, and the cylinder was removed after four days. A third branch was treated similarly but no inoculum was placed in the slit. On May 7, 1934, one of the inoculated branches had conidial fruiting bodies of *Pezicula carpinea* scattered along the branch for about 2 ft. on either side of the point of inoculation. The other inoculated branch and the check were healthy. Some of these conidial fruiting bodies were collected (Univ. of Tor. Herb. *7394*) and the fungus was isolated from the conidia. On Oct. 14, 1934, mature apothecia were thickly scattered along the branch for a distance of about 4 ft. from the point of inoculation, and the infection had evidently spread into the main trunk, as the entire upper part of the tree was dead. There were no fruiting bodies on the other inoculated branch or on the check, but both these branches were dead, having been involved in the death of the tree. The fungus was also isolated from ascospores (Univ. of Tor. Herb. *7268*).

Establishment of the genetic connection between *Pezicula carpinea* and *Cryptosporiopsis fasciculata* by cultural methods has merely confirmed a connection that has long been postulated on the basis of their close association. The conidial stage has been well described by Petrak (12) who observed a variation in which the stroma was less erumpent and evidently more like the conidial fruiting body of *Pezicula acericola* (Pk.) Sacc., described by Groves (6). The writer has not observed this condition in *P. carpinea*, but since this is the typical form of conidial stroma found in the genus, it would not be surprising to find this species sometimes taking this form. The few observations the writer was able to make of the formation of the conidial stroma on agar indicated that the spores were formed first in a cavity in the stroma as in other *Pezicula* species, but that the cavity soon spread open widely, wholly exposing the spore masses.

Some attempt has been made here to bring together the various synonyms of *P. carpinea*. The fungus known as *Ombrophila paradoxa* (Hedw.) Sacc. has been included on the strength of Tulasne's statement (21) that he considered it to be *P. carpinea*. Rehm (15) also mentioned it as a synonym. It would seem that *Peziza Betuli* Alb. and Schw. was based on the conidial stage of *P. carpinea*. The descriptions by Albertini and Schweinitz (1),

Fries (4), and Rehm (14) all omit mention of asci, and von Höhnel (7) recognized it as an imperfect fungus and referred it to the genus *Tuberculariella*. The writer has been unsuccessful in locating the type of *Dermatella scotinus* Morg., but there is nothing in the description to exclude it from *P. carpinea*.

Pezicula pruinosa Farlow, Mycologia 14 : 102. 1922.

Dermatea pruinosa Petrak, Ann. Myc. 20 : 196. 1922.

Sphaeronema pruinosum Peck, Ann. Rep. N.Y. St. Mus. 24 : 85. 1872.

Lagynodella pruinosa Petrak, Ann. Myc. 20 : 207. 1922.

Apothecia (Fig. 2 : 1) strongly erumpent, usually in long rows, sometimes single but usually in elongated clusters, circular, substipitate, 0.5–1.5 mm. in diameter, 0.5–1.0 mm. in height, pale yellow to orange yellow, darker toward the base, slightly pruinose above, becoming strongly greyish-pruinose toward the base, brittle, waxy in consistency, becoming more fleshy when moist; hymenium plane to slightly convex, pruinose when dry, margin paler, slightly raised at first, later disappearing; tissue of the hypothecium compact, pseudo-parenchymatous, often with host cells intermingled, composed of hyaline cells about 5–12 μ in diameter, sometimes more elongated up to 20 μ, arranged in more or less oblique rows toward the outside and thicker walled, and with a granular encrustation on the outside of the apothecium; subhymenium a zone of rather loosely interwoven, slightly coloured hyphae; asci cylindric-clavate, short-stalked, eight-spored, (75)–90–110–(130) $\times$ (12)-14-17–(20) μ; ascospores oblong-ellipsoid to ovoid, hyaline, sometimes slightly coloured, one-to four-celled, occasionally muriform, straight or slightly curved, irregularly biseriate, (14)-17-25–(30) $\times$ (6)–7-9–(10) μ; paraphyses hyaline, filiform, septate, branched, 2.0–2.5 μ in diameter, swollen at the tip up to 5 μ and forming a slight epithecium. (Fig. 4 : 4b.)

Conidial fruiting bodies (Fig. 2 : 2) erumpent, scattered or more or less in rows, usually separate, sometimes with two or three arising from the same basal stroma, cylindric-conic, 0.5–2.0 mm. in height, 0.2–0.4 mm. in diameter at the base and tapering to 150–300 μ at the tip, greyish-pruinose, hard, horny in consistency, becoming more fleshy when moist, the conidia emerging through an irregular opening at the tip or side of the tip of the beak; tissue of the basal stroma composed of thick-walled cells, in the central part almost iso-diametric, 5–10 μ in diameter, toward the outside more elongated, 12–20 $\times$ 5-8 μ, and curving upward in parallel rows, the walls of the beak about 50–75 μ in diameter, consisting of two zones, an outer zone of dark, thick-walled, almost isodiametric cells, and an inner zone of lighter coloured, more elongated cells in parallel rows; conidiophores hyaline, continuous or septate, sometimes branched, 12–30 $\times$ 2.0–2.5 μ, swollen below the tip up to 5 μ, lining the beak and the elongated to ovoid cavity in the basal stroma; conidia oblong-ellipsoid, hyaline, one-celled, straight or slightly curved, ends rounded, one end with a truncate apiculus, 15–25 $\times$ 7–10 μ; microconidia have not been observed. (Fig. 4 : 4a.)

HOST: *Amelanchier* species.

TYPE: Rel. Farl. *135b.*

EXSICCATI: Rel. Farl. *135a, b;* Fung. Dak. *450.*

SPECIMENS EXAMINED: University of Toronto Herbarium. On *Amelanchier canadensis* (L.) Medic. *3407 (14), 3507, 4513, 7987 (319), 7988 (394),* Temagami Forest Reserve, Ontario— ex herb. D. H. Linder, Wellesley, Mass., coll. D. H. Linder, June 5, 1935—ex Cornell Univ. Pl. Path. Herb. *24741,* Lloyd Preserve, McLean, N.Y.

Herbarium of J. W. Groves. On *Amelanchier intermedia* Spach. *263,* McLean Swamp, Ithaca, N.Y., comm. G. E. Thompson.

On *Amelanchier* sp. *438,* Kingsmere, Quebec—*616,* Duchesnay, Quebec.

On malt extract agar the colonies reach a diameter of 3.5–4.5 cm. in two weeks. The mycelium is at first almost colourless, closely appressed to the agar, and somewhat sodden; later whitish to pale grey-green, aerial mycelium develops in unevenly distributed tufts. The conidial fruiting bodies develop as rounded or conical, fleshy stromata, up to 2 mm. in diameter and 3 mm. in height, pale greenish to brownish, and usually covered with white, downy to cottony mycelium, and containing one or more rounded or irregularly lobed cavities, tearing open irregularly at the top or sides. They are composed of interwoven, hyaline or slightly yellowish hyphae, 2–4 μ in diameter, the cavities surrounded by a compact, narrow zone about 25–50 μ in thickness, and sometimes darker coloured. The conidiophores may be longer and more branched than in nature. The conidia are typical. Microconidia are usually abundant, hyaline, filiform, straight or curved, one-celled, 6–10 × 1.5–2.0 μ.

On twigs of *Amelanchier* there is usually more or less abundant, white, cottony, aerial mycelium which forms tufts around the point of inoculation or may spread over the twig. The conidial fruiting bodies (Fig. 3 : 3) develop better when there is less aerial mycelium. They are erumpent, more or less in rows, usually irregularly rounded, up to 1 mm. in diameter and about the same in height, splitting open irregularly at the top or side, but sometimes developing a cylindric-conic ostiole which is usually shorter than in nature, fleshy, drying hard and horny, greyish-pruinose, black or greenish at the tip. The tissue structure is similar to that found in nature, and the conidiophores and conidia are typical.

Establishment by cultural methods of the genetic connection between *Pezicula pruinosa* and *Sphaeronema pruinosum* is also merely the confirmation of a previously suggested relationship. This connection was suggested by Farlow in his original description published by Thaxter (19), and also by Sydow and Petrak (18). The conidial stage was collected in the Temagami region in the spring of 1932 when this study was commenced, but although search was made for it, the perfect stage was not found until 1935. In June, 1934, a tree with abundant conidial fructifications was selected and kept under observation until the middle of September, but no apothecia were produced. The

following year it was again kept under observation, and on July 2, 1935, mature apothecia were found.

The chief distinguishing characters of this species are its occurrence on *Amelanchier*, the yellow, waxy-fleshy, substipitate apothecia which are markedly greyish-pruinose near the base, and the *Sphaeronema*-like conidial stage. The conidial stage was first described by Peck (10) as a *Sphaeronema*, and Petrak (18) made it the type of his new genus *Lagynodella*. It is doubtful if *Sphaeronema pruinosum* Berk. and Curt. described on *Rhus venenata* is the same fungus.

Pezicula Corni Petrak, Ann. Myc. 20 : 197. 1922.

> *Dermatea Corni* Phill. and Hark., Grev. 13 : 22. 1884.
>
> *Ocellaria aurea* var. *cornicola* Kauffm. and Kanouse, Papers Mich. Acad. Sci., Arts, and Letters, 9 : 183. 1929.
>
> *Sphaeropsis cornina* Peck, Ann. Rep. N.Y. St. Mus. 32 : 38. 1879.
>
> *Macrophoma cornina* Sacc., Syll. Fung. 10 : 192. 1892.
>
> *Cryptosporiopsis cornina* Petr. and Syd., Ann. Myc. 22 : 370. 1924.

Apothecia (Fig. 1 : 5) erumpent, sub-immersed when dry, becoming more strongly exserted when moist, scattered or more or less in rows, separate, sometimes in small clusters, circular, sessile, slightly narrowed below, 0.4–1.0 mm. in diameter, 0.2–0.4 mm. in height, ochraceous yellow to dull reddish-orange, slightly pruinose above, becoming darker to almost black below, soft, waxy, crumbling easily, more fleshy when moist; hymenium plane to convex, slightly pruinose, with a slight margin; tissue of the hypothecium compact, pseudoparenchymatous, composed of slightly coloured, irregular, angular cells about 5–15 μ in diameter, arranged in more or less vertically parallel rows curving obliquely toward the outside, golden yellow in the central part, becoming much darker toward the outside, cells more elongated in the upper part; subhymenium a zone of closely interwoven hyphae; asci broadly clavate or subellipsoid, short-stalked, eight-spored, (85)–105–130–(145) $\times$ (20)–23–30-(33) μ; ascospores oblong-ellipsoid to ovoid, hyaline or slightly yellowish, one- to four-celled, straight or slightly curved, irregularly biseriate, (22)–25–35–(40) $\times$ (10)–12–15–(17.5) μ; paraphyses hyaline, filiform, septate, simple or branched, 2.0–3.0 μ in diameter, the tips often coloured and swollen up to 5 μ, forming a slight epithecium. (Fig. 4 : 2b.)

Conidial fruiting bodies erumpent, scattered, mostly separate, occasionally two or three together, black, circular, rounded to conical, opening widely and irregularly, 200–350 μ in diameter, 300–450 μ in height, containing a single, slightly lobed cavity; the basal stroma a pseudoparenchymatous layer 25–35 μ in thickness, composed of dark, thick-walled cells, 5–8 μ in diameter, in the central part the stroma usually much thicker, up to 100 μ, and the tissue composed of lighter coloured, closely interwoven hyphae; conidiophores hyaline, continuous or sometimes septate, simple or branched, cylindric or conical, sometimes swollen slightly below the tip, 10–30 $\times$ 3–6 μ; conidia

oblong-ellipsoid to ovoid, hyaline or slightly yellowish, straight or slightly curved, one- to four-celled, ends rounded, one end with a truncate apiculus, (25)–30–35–(40) × (9)–11–13–(15) μ; microconidia hyaline, filiform, curved or sometimes almost straight, one-celled, 12–18 × 1.5–2.0 μ. (Fig. 4 : 2a.)

HOST: *Cornus* species.

TYPE: On *Cornus stolonifera* Michx. Priest River, Bonner Co., Idaho, J. R. Weir, *16909*. Oct. 11, 1918.

EXSICCATI: Ell. N. Am. Fung. *2809* (as *Pezicula rhabarbarina*).

SPECIMENS EXAMINED: University of Toronto Herbarium. On *Cornus stolonifera* Michx. *4833 (138)*, Toronto, Ontario—ex U.S. D. A. Path. and Myc. Coll. Herb. J. R. Weir *16909*, part of type—ex Farl. Herb. Harvard Univ., coll. G. D. Darker *(5484)*, Waltham, Mass.

On *Cornus circinata* L'Her. *4836 (126)*, *4839 (129)*, Ottawa, Ontario— *6564 (280)*, Aurora, Ontario—*5699*, *6590*, *(243)*, *7974*, *7975 (449)*, *7976*, Temagami Forest Reserve, Ontario.

On *Cornus alternifolia* L. Ex Univ. of Mich. Crypt. Herb. Part of type of *Ocellaria aurea* var. *cornicola* Kauffm. and Kanouse, Rock River, Mich. Sept. 4, 1927.

On *Cornus* sp. *6940*, Aurora, Ontario—*7977*, Temagami Forest Reserve, Ontario—ex herb. L. O. Overholts *13370*, Hawn's Bridge, Hunt. Co., Pa.

Herbarium of J. W. Groves. On *Cornus* sp. *553*, *554*, Casey's Corners, Nova Scotia—*584*, Ottawa, Ontario.

Harkness No. *2259*, the type of *Dermatea Corni* Phill. and Hark. A single apothecium in the Durand Herbarium of Discomycetes, Cornell University *3786*, and a slide from the specimen in Kew Herbarium.

On malt extract agar the colonies reach a diameter of 2–3 cm. in three weeks. They are whitish to greenish-grey, sometimes slightly yellowish to orange at the centre, the aerial mycelium loose and fluffy, then becoming slightly matted. The conidial fruiting bodies are almost globose to sub-cylindric, dark greenish to almost black, fleshy, glabrous or covered with a short, grey mycelium, about 150–350 μ in diameter, sometimes larger, tearing open widely and irregularly at the top, and containing a single, rounded or somewhat lobed cavity; tissue composed of closely interwoven, brownish hyphae, 2–5 μ in diameter; conidiophores, conidia, and microconidia as found in nature.

On twigs of *Cornus* there is usually very little aerial mycelium, but sometimes greenish or brownish tufts appear at the point of inoculation. The twigs become thickly covered with small, black, erumpent stromata, more or less in rows, at first rounded and covered with short, reddish-brown, hair-like hyphae, then sometimes elongating and becoming cylindric-conic, opening widely at the top. These fruiting bodies (Fig. 2 : 3) are very similar in structure to the fruiting bodies as found in nature, and produce typical conidia and microconidia.

The chief distinguishing characters of this species are its occurrence on *Cornus*, the ochraceous-orange, waxy apothecia, not strongly erumpent and usually single, the broad asci, large spores, and the *Macrophoma*-like conidial stage.   As far as is known, the genetic connection of *Pezicula Corni* and *Cryptosporiopsis cornina* has not been reported previously.   The *Myxosporium* species reported by Seaver (17) as the conidial stage of this species is undoubtedly *C. cornina*.   This species has been confused to some extent with *Pezicula Rubi* (Lib.) Niessl, from which it can readily be distinguished by the size of the asci and spores and the type of conidial stage.   Probably all the records of *P. Rubi* on *Cornus* will prove to be *P. Corni*.

Seaver (17) has drawn attention to the fact that the identity of *Dermatea Corni* Phill. and Hark., an earlier name, is uncertain.   The writer has made some effort to locate the type of this species, which should be Harkness' No. *2259* according to the original description.   In the Durand Herbarium of Discomycetes at Cornell University No. *3786*, is a packet labelled *Dermatea Corni* Phill. and Hark. Co-type, and bearing the above Harkness number. This packet contains a single apothecium detached from the substrate, but which is unquestionably a *Pezicula*, and is similar to *P. Corni* Petr. in gross appearance.   Some of the writer's material was sent to Mr. E. W. Mason of the Imperial Mycological Institute, with the request that he compare it with the type material of *Dermatea Corni* if any were to be found in the Phillips Collection.   Mr. Mason kindly did this and reported that the specimen in the Phillips Collection was very scanty and seemed mostly immature, but was evidently the same fungus.   Examination of a slide which Mr. Mason loaned to the writer showed that although the fungus was immature, some of the characteristic large asci and spores were present.

The writer is, therefore, convinced that *Dermatea Corni* Phill. and Hark. is identical with *Pezicula Corni* Petr.   The fungus is a *Pezicula*, but since Petrak has used the combination *P. Corni*, it would now be necessary to give the fungus a new name in order to conform strictly to the rules.   However, in view of the fact that the type material of *Dermatea Corni* is scanty and immature, and that Petrak's name is established in the literature and is eminently suitable, it would seem preferable to retain this name.

Pezicula Rubi (Lib.) Niessl, Rabh. fung. Eur. n. 2122.   1876.

> *Patellaria Rubi* Libert, Pl. Crypt. Ard. n. 231.   1834.
>
> *Helotium Rubi* Spree, Rabh. fung. Eur. n. 717.   1865.
>
> *Dermatea Rubi* Rehm, Rabh. Krypt. Fl. I, 3 : 258.   1889.
>
> *Peziza arduennsis* Montg., Ann Sci. Nat. II, T. V. p. 287, t. 13, f. 5. 1836.
>
> *Gloeosporium phaeosorum* Sacc., Mich. I : 360.   1878.
>
> *Myxosporium phaeosorum* Allescher, Rabh. Krypt. Fl. I, 7 : 529.   1902.
>
> *Discosporium phaeosorum* v. Höhn., Z. Gärungsphysiol. 5 : 197.   1915.
>
> *Discosporiella phaeosora* Petrak, Ann. Myc. 21 : 14.   1923.

Apothecia (Fig. 2 : 4) erumpent, scattered, separate or sometimes two or three in a cluster, circular, sessile, narrowed below, 0.2–1.0 mm. in diameter, 0.2–0.5 mm. in height, dull ochraceous yellowish to reddish orange, brighter when moist, slightly pruinose, brittle, waxy in consistency, more fleshy when moist; hymenium at first concave, then plane to convex, slightly pruinose, margin at first raised, later disappearing; tissue of the hypothecium pseudo-parenchymatous, at the base composed of hyaline to yellowish-brown, thick-walled cells, 3–8 μ in diameter, more elongated above and somewhat inter-woven, toward the outside in obliquely parallel rows, dark, and thick-walled, 5–12 μ in diameter, sometimes the outer cells projecting slightly and clavate; subhymenium a narrow zone of closely interwoven hyphae; asci cylindric-clavate, more or less stalked, eight-spored, (75)–85–100–(115) × (10)–12–15–(17) μ; ascospores oblong-ellipsoid to ovoid or subfusiform, hyaline, straight or slightly curved, one- to four-celled, irregularly biseriate, (15)–18–25–(30) × 5–8 μ; paraphyses hyaline, filiform, septate, simple or branched, 1.5–2.0 μ in diameter, swollen at the tips up to 5–6 μ, or sometimes only slightly swollen, and forming a slight epithecium. (Fig. 4; 3b.)

Conidial fruiting bodies (Fig. 1 : 3) thickly scattered, developing below or within the epidermis and splitting it, forming little blisters but scarcely breaking through, 150–300 μ in diameter, circular or elliptical, consisting of a thin basal layer 5–8 μ in thickness, composed of hyaline, indistinct hyphae from which the conidiophores arise; conidiophores hyaline, continuous or septate, not observed branching, conical or cylindric, sometimes slightly swollen below the tip, 5–12 × 1.5–2.0 μ; conidia oblong-ellipsoid to elongated, hyaline or pale yellowish-green, one-celled, straight or slightly curved, ends rounded or slightly tapering, one end with a truncate apiculus, (15)–20–28–(33) × (5)–6–7.5–(8.5) μ; no microconidia observed. (Fig. 4 : 3a.)

Host: *Rubus* species.

Type: Libert Pl. Crypt. Ard. Fasc. III, *231.*

Exsiccati: Rel. Farl. *114*; Fung. Col. *1120* (as *Pezicula rhabarbarina*); Kr. Fung. Sax. *41, 1146.*

Specimens Examined: University of Toronto Herbarium. On *Rubus* species. *6813 (270), 6843 (283),* Toronto, Ontario—*6960 (276),* Bell's Lake, north of Parry Sound, Ontario—*7403 (292),* Mt. Nemo, north of Nelson, Ontario—*7990 (419),* Temagami Forest Reserve, Ontario.

Herbarium of J. W. Groves. On *Rubus* species. *505,* Graham Bay, Ontario—*622,* Constance Bay, Ontario.

Durand Herbarium of Discomycetes. *3666,* ex Lib. Pl. Crypt. Ard. *231.* part of type.

On malt extract agar the colonies reach a diameter of 4.5–5.0 cm. in two weeks. The mycelium is sodden and closely appressed, with scant, fluffy to matted, more or less tufted, whitish to slightly pinkish, aerial mycelium. The conidial fruiting bodies are rounded, almost globose, black or greenish-black, usually covered with a white, downy mycelium, 0.2–3 mm. in diameter,

containing one or sometimes several, rounded or lobed cavities, tearing open widely and irregularly. The tissue is composed of hyaline, closely interwoven hyphae, about 2-3 μ in diameter, looser toward the outside. The cavity is surrounded by a zone of compact, pseudoparenchymatous tissue, composed of small, irregular cells, 3-7 μ in diameter. The conidiophores and conidia are typical. Microconidia present, hyaline, filiform, one-celled, straight or curved, 10-30 $\times$ 1.5-2.0 μ.

On twigs of *Rubus* the aerial mycelium is usually not abundant, forming a white or buff, cottony tuft at the point of inoculation, and occasionally spreading over the twig. The conidial fruiting bodies (Fig. 3 : 4) are erumpent, breaking through the epidermis and developing chiefly on the surface of the twig, rounded to cylindric, 0.3-1.0 mm. in diameter, 0.2-0.6 mm. in height, brownish to blackish, usually covered with a loose, whitish to brownish mycelium. The basal stroma may remain thin as in nature, or may develop to a thickness of 250 μ and contain one or more cavities in the upper part. The tissue is composed of closely interwoven hyphae, looser at the outside, sometimes pseudoparenchymatous at the base. The conidia and conidiophores are typical.

In one twig culture originating from mass ascospores, several apothecia were produced (Fig. 1 : 4). They were typical of the apothecia as found in nature in both macroscopic and microscopic characters, and cultures from the ascospores were typical.

Some confusion has existed regarding the specific identity of this fungus, and apparently the name has been applied to more than one species. Evidently the type may be considered to be the specimen issued in Pl. Crypt. Ard. Fasc. III, No. *231*, and a small fragment of this specimen in the Durand Herbarium of Discomycetes at Cornell University No. *3666*, was examined. This packet bore the label "*Patellaria Rubi* Lib. on fallen branches of *Rubus*" and contained only two apothecia detached from the substrate, but they were evidently the same fungus that occurs on *Rubus* in this region, and the determination of the writer's material is based on this specimen and also that issued in Kr. Fung. Sax. *41*.

The chief distinguishing characters of this species are its occurrence on *Rubus*, the ochraceous, waxy, mostly single apothecia, narrow asci and spores, and the *Gloeosporium*-like conidial stage. The genetic connection of *Pezicula Rubi* and *Discosporiella phaeosora* was suggested by Petrak (11), and this has been confirmed by the cultural studies above. Petrak (11) based the genus *Discosporiella* on this species, characterizing it as a *Discosporiopsis* (= *Cryptosporiopsis* Bub. & Kab.) with a very thin basal stroma. Nannfeldt (8) took the view that this was not a satisfactory character on which to base a generic separation, and in the writer's cultures the basal stroma has varied from 10-250 μ in thickness, thus supporting Nannfeldt's contention.

The writer is of the opinion that *Pezicula Rubi* occurs only on *Rubus*, although it has been reported on other hosts. The type of *P. Rubi* was on *Rubus* as noted above. Berkeley (2) described a fungus as *Peziza rhabarbarina*,

which he said occurred on wild rose, and which Tulasne (21) later transferred to *Pezicula*. Saccardo (16) also described a *Pezicula* occurring on rose as *Pezicula Rosae*. Phillips (13) considered *P. Rosae* to be a synonym of *P. rhabarbarina* and *P. Rubi* to be distinct; but Rehm (14) considered *P. rhabarbarina* to be a synonym of *P. Rubi* and *P. Rosae* to be distinct. Rehm gave the host of *P. Rubi* as "an dürren Ranken von *Rubus fruticosus*, seltener von wilder *Rosa*", and of *P. Rosae* as "an dürren Aesten von *Rosa canina*". In addition *P. Rubi* has been reported on *Cornus* and *Hamamelis*. The cultural and morphological studies reported in this paper have demonstrated that there are three distinct species occurring on *Rubus*, *Cornus* and *Hamamelis*, and that these three species can be separated in gross appearance, microscopic characters, cultural characters, and especially by the type of conidial stage. Examination of all available collections and exsiccati has, so far, confirmed this separation on the basis of hosts. The question as to whether *P. Rubi* may occur also on rose must remain undecided for the present. The writer has had no opportunity of examining fresh material of the fungus on rose, and its conidial stage is unknown, but examination of the specimen in Brenckle Fung. Dak. *392*, labelled *Pezicula Rosae* Sacc., shows that it is evidently distinct from all three of the above species. The writer is of the opinion, therefore, that Phillips' interpretation is more probably the correct one and that these fungi are more closely restricted to the host than has been considered heretofore. It is not inconceivable that *P. Rubi* might occasionally occur on rose, but since there evidently is a distinct species occurring on rose, reports of the occurrence of *P. Rubi* on this host should be supported by cultural evidence showing the type of conidial stage.

Nannfeldt (9) concluded from an examination of the type specimen of *Helotium scoparium* Cke. that it was only *Pezicula Rubi* in a bad state of preservation. The writer would also question this determination, on the grounds of host relationship. *H. scoparium* was described on *Sarothamnus* of the Leguminosae, and the occurrence of *P. Rubi* on this host would seem most unlikely.

Pezicula Hamamelidis Groves and Seaver sp. nov.

Apotheciis erumpentibus, dispersis vel plus minus seriatim instructis, solitariis vel caespitosis, sessilibus, versus basim attenuatis, orbicularibus, 0.3–1.0 mm. latis, 0.2–0.4 mm. altis, ochraceo-flavis, mollibus, in sicco ceraceis, in humido carnosis; hymenio plano vel convexo, leviter pruinoso, margine primo pallido dein evanescente; hypothecio pseudoparenchymato; ascis oblongo-clavatis, brevissimo stipitatis, octosporis, 90–120 × 20–25–(30) μ; ascosporis elliptico-oblongis vel ovoideis, hyalinis, rectis vel leviter curvatis, continuis vel triseptatis, (20)–25–33–(37.5) × (8)–9–11–(13) μ; paraphysibus hyalinis, filiformibus, septatis, simplicibus vel ramosis, 2–3 μ diam., apice ad 8 μ incrassatis, leve epithecium formantibus.

Apothecia (Fig. 3 : 1) erumpent, scattered or more or less in rows, usually single, sometimes caespitose with two to six in a cluster, sessile, narrowed below, circular, 0.3–1.0 mm. in diameter, 0.2–0.4 mm. in height, pale ochraceous yellowish, close to cinnamon buff when dry, brighter, close to

warm buff when moist, brittle, waxy in consistency, more fleshy when moist; hymenium plane or convex, slightly pruinose, at first with a delicate, pale border which later disappears; tissue of the hypothecium compact, pseudo-parenchymatous, composed of thick-walled, more or less isodiametric to somewhat elongated cells about 5–10 μ in diameter, hyaline to golden yellow, arranged in more or less vertically parallel rows which curve obliquely toward the outside where they are darker coloured; subhymenium narrow, composed of rather loosely interwoven hyphae about 3–5 μ in diameter; asci oblong-clavate, broad, short-stalked, eight-spored, 90–120 $\times$ 20–25–(30) μ; ascospores oblong-ellipsoid to ovoid, hyaline, straight or slightly curved, one- to four-celled, irregularly biseriate, (20)–25–33–(37.5) $\times$ (8)–9–11–(13) μ; paraphyses hyaline, filiform, septate, simple or occasionally branched, 2–3 μ in diameter, the tips clavate and swollen up to 8 μ in diameter, forming a slight, yellowish epithecium. (Fig. 4 : 5b.)

Conidial fruiting bodies minute, about 300–450 μ in diameter, developing beneath the outer bark layers and splitting them slightly, the conidia emerging in whitish masses. The fruiting body consists of a cushion-shaped basal stroma, usually much thicker in the centre than at the periphery, and containing one or possibly more cavities; tissue of the basal stroma pseudo-parenchymatous, composed of hyaline, irregular cells about 3–8 μ in diameter, becoming more elongated and interwoven in the thicker central part, the cavities surrounded by a narrow zone of hyaline, slender, indistinct hyphae; conidiophores hyaline, cylindric, septate, simple or sometimes branched, 15–30 $\times$ 2.5–3 μ, swollen below the tip up to 4 μ; conidia oblong-ellipsoid, hyaline, one-celled, straight or slightly curved, one end with a truncate apiculus, (25)–30–37–(45) $\times$ (9)–12–14.5 μ; microconidia borne in the same fruiting body, hyaline, filiform, straight or curved, one-celled, 10–18 $\times$ 1.5–2.0 μ. (Fig. 4 : 5a.)

HOST: *Hamamelis virginiana* L.

TYPE: University of Toronto Herbarium *7983*, Don Valley, Toronto, Ontario. Coll. J. W. Groves, Oct. 26, 1935.

EXSICCATI: Syd. Fung. exot. exs. *423* (as *Dermatea Rubi*).

SPECIMENS EXAMINED: University of Toronto Herbarium. On *Hamamelis virginiana* L. *2755, 4373 (54), 4375 (55), 4380 (59), 7982, 7983 (443)*. Toronto, Ontario—*7984*, Brant Co., Ontario.

Herbarium of J. W. Groves. On *Hamamelis virginiana. 56, 272*, Toronto, Ontario.

On malt extract agar the colonies reach a diameter of about 4 cm. in two weeks, producing a white to pale yellowish or brownish, loosely matted, to fluffy or cottony aerial mycelium. Conidial stromata are formed only occasionally and are usually much larger than in nature, up to 2 mm. in diameter and 1 mm. in height, more or less rounded, fleshy, buff, brownish, or olivaceous, becoming covered with a white, downy mycelium, at first with drops of clear green or amber liquid on them, then splitting open widely and irregularly,

exposing the whitish spore masses; the tissue composed of hyaline, rather loosely interwoven hyphae, 2.5–4.0 μ in diameter, with a narrow, compact zone of closely interwoven hyphae surrounding the cavity. The conidia and conidiophores are typical and microconidia are very abundant.

On twigs of *Hamamelis* a thin, white, cottony, aerial mycelium is sometimes formed but is usually not abundant. The conidial fruiting bodies (Fig. 3 : 5) are not formed abundantly and sporulation is rare. They are usually strongly erumpent, rounded or irregular in shape, sometimes subcylindric, covered with a white, downy mycelium. The tissue of the stroma is pseudoparenchymatous at the base, composed of hyaline, irregular cells, 5–15 μ in diameter, becoming more prosenchymatous in the upper part, the hyphae rather loosely interwoven but with a compact, yellowish zone, 20–40 μ in thickness, surrounding the cavity. Microconidia are usually abundant while macroconidia are only occasionally produced.

This species was collected near Toronto by Professor H. S. Jackson, and sent to Dr. F. J. Seaver before the writer had begun the study of this group. Dr. Seaver recognized it as an undescribed species, proposing the manuscript name *Pezicula Hamamelidis*, and subsequently suggested, when this cultural study was made, that the writer describe the species.

The chief distinguishing characters of this fungus are its occurrence on *Hamamelis*, the ochraceous, waxy, mostly single and not strongly erumpent apothecia, broad asci, and the *Cryptosporiopsis* type of conidial stage. The asci are usually shorter and the ascospores narrower than in *Pezicula Corni*, and both asci and ascospores are broader than in *P. Rubi*. It has evidently been confused with the latter in the past, but it can readily be distinguished by the size of the asci and ascospores as well as by the size of conidia and type of conidial fruiting body. Cultures of the two forms are quite different in appearance also, and there is no doubt that they are distinct species.

The conidial fruiting bodies are extremely inconspicuous, and were only discovered by placing twigs bearing young apothecia in a moist chamber and observing the masses of conidia which emerged through tiny cracks in the bark. As far as could be ascertained the conidial stage is also undescribed, but it is a typical *Cryptosporiopsis*, and the majority of *Pezicula* species which the writer has studied have had conidial stages of essentially the same form as that found in *P. Hamamelidis*.

Conclusion

In these five species, all belonging to the genus *Pezicula*, we thus find conidial stages that could be referred to widely separated families of the Fungi Imperfecti; for example, the conidial stage of *P. carpinea* could be referred to the Tuberculariaceae, of *P. pruinosa* to the Phomaceae, and of *P. Rubi* to the Melanconiaceae. In culture, although retaining some of the characters as found in nature, these conidial stages nevertheless tend to approach a common form, which consists of a more or less globose stroma containing one or more cavities. This is probably the primitive type of stroma in this genus, from

which the other types have evolved. It is of interest to note that in spite of the range of variation in the conidial stroma, the conidial spore itself remains rather constantly oblong-ellipsoid in form. The form of the spore in this group may, therefore, be considered of greater importance in indicating relationships than the form of the stroma. This will be more fully discussed after species of the related genus *Dermatea* have been considered.

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. BULB NEMATODE CONTROL IN IRIS BY HOT WATER[1]

BY R. J. HASTINGS[2], J. E. BOSHER[3] AND WILLIAM NEWTON[4]

Abstract

The immersion of iris bulbs in water at 110 to 112° F. for 60 min. effectively destroys the bulb nematode.

The safe period for nematode destruction in iris by hot water lies between July 26 and August 9. Thereafter injury to the bulbs occurs and is progressively greater as the immersion dates are delayed.

Introduction

The hot water treatment is an efficient method for the control of the bulb nematode in narcissi when the bulbs are immersed for three hours at 110 to 112° F. shortly after they are lifted.

A three-hour immersion period was found to be highly injurious to iris bulbs, hence a study was undertaken to ascertain whether a shorter immersion period might destroy the bulb nematode in iris without injuring significantly the vitality of the bulbs. At the outset, there appeared to be little hope that a shorter immersion period would prove effective, for there is little evidence that a distinct strain is involved in iris parasitism (4). Sherman (5) and Hastings and Newton (3) have reported bulb nematode survivals in narcissus after an immersion for three hours at 110 to 112° F. These survivals were usually pre-adults. In narcissus the pre-adult stage predominates in the nematode populations after long storage periods. Hastings and Bosher (2) have analyzed the nematode populations in a few varieties of iris and have reported small populations that consist largely of young larvae. Also, where large populations were found, the analyses revealed that they were principally young larvae. As our studies of the heat resistance of mixed stage cultures (3) showed that young larvae are destroyed within 60 min. at 110 to 112° F., it seemed probable that a 60-min. immersion period would prove effective in the control of the bulb nematode in iris, providing the bulbs themselves were not significantly injured by the heat treatment.

The English and American experiments covering the effect of hot water immersion upon iris have been briefly reviewed by Goodey (1) in his presidential address to the Association of Applied Biologists in 1936. He stated that the attempts of Steiner and Buhrer (Washington, D.C.) and Hodson (England) to control the bulb nematode in iris by hot water were not successful. On the other hand, he stated that Staniland successfully controlled the

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nematode in "Imperator" bulbs by immersing them in early autumn for 50 min. at 110 to 112° F.

In correspondence, Dr. F. Weiss reports that in 1936 a quantity of North Carolina iris bulbs were put through a one- to three-hour immersion in water at 110 to 112° F. without injury, between July and the end of August, but severe injury occurred when iris bulbs were similarly treated in mid-October. Dr. Weiss states that the bulbs grown in the southeastern United States attain a greater degree of dormancy than those grown in the northwest.   It is probable therefore that the safe immersion period in British Columbia may be shorter than in North Carolina.

Experimental

Parcels of "Supreme", "Imperator", and "Wedgewood" iris bulbs were immersed in water at 110 to 112° F. for one hour at fortnightly intervals between July 26 and October 19, in 1937.   After they had been removed

TABLE I

THE EFFECT OF A ONE-HOUR IMMERSION OF IRIS BULBS IN WATER AT 110 TO 112° F. ON BULB AND NEMATODE SURVIVAL AS INFLUENCED BY THE DATE OF TREATMENT

Variety	Date of treatment	Planted weight, gm.	Harvested weight, gm.	Percentage infested with bulb nematode
Supreme	Check	1000	837	94
	Check	1000	602	96.2
	26 July	1000	1340	0
	9 August	1000	1436	0
	23	1000	1133	0
	9 September	1000	626	0
	20	1000	336	0
	19 October	1000	184	0
Imperator	Check	1000	1625	0*
	Check	1000	1454	0
	Check	1000	1410	0
	26 July	1000	1733	0.
	9 August	1000	1684	0
	23	1000	1463	0
	9 September	1000	1335	0
	20	1000	1320	0
	19 October	1000	640	0
Wedgewood	Check	1000	1625	0
	Check	1000	1485	0
	Check	1000	1442	0
	26 July	1000	1757	0
	9 August	1000	1671	0
	23	1000	1465	0
	9 September	1000	1367	0
	20	1000	1350	0
	19 October	1000	670	0

* *The Imperator and Wedgewood bulbs were included to ascertain their physiological response to hot water.   The planting stock was slightly infested but no nematodes could be found in the harvested crop, irrespective of treatment.*

from the hot water bath, they were immersed in a 0.0015% suspension of mercury as methyl mercury nitrate* for 30 min., then placed in storage. All parcels were planted in the field on November 4. The immersion in an organic mercury suspension was deemed essential as a protection against *Penicillium* rots, which normally cause considerable injury; and susceptibility to *Penicillium* injury is increased by hot water treatments.

The results are given in Table I. It is readily seen from the weight of the bulbs harvested that injury occurs unless the bulbs are lifted early and immersed shortly after. In all three varieties, injury occurred after August 23 and became progressively greater as the immersion periods were delayed. It is apparent that a one-hour treatment destroys the majority of the nematodes. No living nematodes were recovered from any lot immersed for 60 min. at 110 to 112° F.

* *Sold under the trade name of "Leytosan", with mercury equivalent to 1.5%.*

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MICROBIOLOGICAL STUDIES OF APPALACHIAN PODSOL SOILS

IV. THE DECOMPOSITION OF GLUCOSE IN SOILS PREVIOUSLY TREATED WITH AMENDMENTS[1]

By P. H. H. Gray[2] and C. B. Taylor[3]

Abstract

A study has been made of the decomposition of glucose in two cultivated podsol soils that had previously been treated with alkaline amendments. Limestone increased, and sodium carbonate decreased, the amount of carbon dioxide produced from the control samples; limestone increased the rate of evolution both in control samples and in samples receiving glucose. The total amount of carbon dioxide produced was increased by limestone in combination with sodium carbonate but not by limestone alone. The numbers of heterotrophic bacteria developing with glucose were stimulated by limestone.

Introduction

It has recently been shown (3) that the addition of alkaline amendments to podsol soils increased total microbial activity, nitrification, and the numbers of bacteria. The significant increases in the three biological factors studied were, by the average of treatments, 62, 181, and 195% respectively. These values are derived from the average of the percentage increases found, less the percentage required for a significant difference, in 10 of the 11 treated plots. The increases suggested that the treatment of field soil with calcium oxide, sodium carbonate, and sodium hydroxide released a considerable amount of readily available food material for the soil micro-organisms. The material released must have contained carbonaceous as well as nitrogenous substances, since the carbon dioxide output as well as the activity of the heterotrophic and the nitrifying bacteria was increased. Some of the carbonaceous material may have been derived from the incompletely decomposed plant residues, and some from that part of the soil organic matter that includes the cells of micro-organisms. Shaw and McKibbin (5) have determined the amount of soluble organic matter that such treatments will liberate from podsol soils under laboratory conditions; it is not known, however, what proportions of the freed material will serve as sources of energy and food for soil organisms.

The ratio of organic carbon to total nitrogen in these soils is about 15 : 1. As the relative increase in activity was least in terms of the soil's total microbial complex, it would seem reasonable to assume that there was relatively less carbonaceous than nitrogenous material liberated by those treatments. It would appear, therefore, that the carbonaceous residues of the soil are in a

form not readily made available, and only partially so after treatment with organic matter solvents such as sodium carbonate. It has been shown that the addition of nitrate of soda alone does not increase microbial activity or bacterial numbers in these soils (3).

It seemed, therefore, that an understanding of the nature of the processes arising from such treatments could be obtained by determining the intensity of microbial activity after adding an easily available carbon compound, such as glucose, to soils previously treated with amendments.

Experimental

NUMBERS OF MICRO-ORGANISMS

The soils selected for study were taken in May 1937 from plots under experiment in the vicinity of East Clifton and Sawyerville, Compton County, Que. The plots were situated on two farms, and had been treated with limestone, sodium carbonate, and a combination of these two amendments, as reported in a previous paper (2). The treatments were applied in October 1935. Their effects on the numbers of bacteria (with actinomyces) and fungi were studied in five samplings during the summer of 1937, when the plots were under clover and timothy grass. The average numbers of micro-organisms in the samplings, determined by plating with Thornton's medium, with soil extract glucose agar for bacteria and actinomyces, and with the latter medium made acid with tartaric acid for the fungi, were as shown in Table I.

TABLE I

NUMBERS OF MICRO-ORGANISMS IN SOILS TREATED WITH AMENDMENTS

Soil treatment	Soil *R*		Soil *T*	
	Thornton's medium	Soil extract agar	Thornton's medium	Soil extract agar
	Bacteria and actinomyces, millions per gm.			
Control	6.93	13.96	12.47	24.18
Limestone	12.04	26.86	35.43	35.74
Sodium carbonate	13.43	27.20	28.28	31.96
Both amendments	14.63	31.80	32.39	35.04
	Fungi, thousands per gm. (av. of 3 samplings)			
Control	—	125	—	147
Limestone	—	88	—	133
Sodium carbonate	—	105	—	115
Both amendments	—	103	—	132

The results given in Table I show that with both mediums used the bacteria were greatly increased by the treatments, though these had been applied 18 to 23 months previously. The sodium carbonate was as effective as the

limestone; both amendments together appear not to have caused any increase over the numbers given by each one separately. The effects of sodium carbonate had thus lasted through two seasons, as shown in the earlier work (3). Soil *T* supported the denser microflora of the two, as shown by comparing the numbers in the control plots. This difference, as previously pointed out (2), was probably due to the fact that this soil had been in the virgin state only two years before. The addition of the amendments to this soil caused a greater increase in the numbers of bacteria growing in the more selective medium, than in the soil extract agar.

The fungi were depressed by the treatments.

NITRIFYING CAPACITY

Samples from these plots were also used to inoculate a mineral salt medium, in order to determine if the treatments had stimulated the nitrifying bacteria to develop a greater capacity to oxidize ammonium sulphate under controlled conditions in the laboratory. Since the nitrifying bacteria prefer a neutral or alkaline environment to enable them to oxidize ammonium compounds most rapidly, it was expected that these treatments might be found to stimulate the rate of nitrification.

The pH values of fresh samples from these plots were determined by the electrometric method (by Mr. H. L. Fletcher) and were found to be as follows:

Treatment	Soil *R*	Soil *T*
Nil	4.98	4.70
Limestone	6 18	6 67
Sodium carbonate	5.63	5 42
Both amendments	6.96	5.71

The medium used for cultivating the nitrifying bacteria was composed of the following salts (1): K_2HPO_4, 1.0 gm.; $MgSO_4 . 7H_2O$, 0.5 gm.; NaCl, 2.0 gm.; $FeSO_4$, 0.4 gm.; $(NH_4)_2SO_4$, 1.0 gm.; water, 1000.0 gm. The medium was dispensed in 25-ml. quantities in Erlenmeyer flasks of 125 ml. capacity, and sterilized. Magnesium carbonate was sterilized in 0.2-gm. quantities in tubes and added to the sterile salts. Three flasks of medium were inoculated with 1 gm. of each soil, three with a garden soil over limestone for comparison, and three were kept as sterile controls. The cultures were incubated at 28° C. and tested at intervals for nitrites and nitrates. Nitrites were tested for by means of Trommsdorf's reagent. After the nitrites had disappeared, the cultures were tested for nitrates with diphenylamine in sulphuric acid. The results are shown in Table II. The oxidation of ammonium sulphate proceeded more quickly, in both soils, with samples from the plots that had received sodium carbonate than with samples from the control plots. The soil with the higher bacterial numbers, Soil *T*, was the more active, though slightly more acid, and treatment with limestone brought it up

to the level of a rich garden soil. The combination of sodium carbonate with limestone stimulated the bacteria in Soil R more than did limestone alone. This test would seem to confirm the view that the treatments with sodium carbonate had released nitrogenous material for the use of the nitrifying

TABLE II

NITRIFICATION OF AMMONIUM SULPHATE IN SOLUTION CULTURES

Soil	Treatment	Duration of experiment, days												
		5	8	11	14	18	22	24	27	31	35	38	41	51
R	Nil	0	0	0	0	0	0	±	±	±	+	+	∓	∓
	CaCO₃	0	0	0	0	±	+	+	+	+	+	+	∓	X
	Na₂CO₃	0	0	0	0	±	±	±	+	+	+	+	+	+
	CaCO₃ } Na₂CO₃	0	0	+	+	+	+	+	+	∓	X			
T	Nil	0	0	0	0	+	+	+	+	+	+	+	X	
	CaCO₃	0	+	+	+	+	+	+	X					
	Na₂CO₃	0	0	0	±	+	+	+	+	+	X			
	CaCO₃ } Na₂CO₃	0	+	+	+	+	+	∓	X					
Garden	—	0	+	+	+	+	∓	X						

0 = *nitrite not yet formed;* ± = *nitrite reaction slight;* + = *nitrite reaction strong;* ∓ = *nitrites decreasing;* X = *nitrites absent, nitrates present.*

bacteria in the soil, enabling them to develop higher numbers and to oxidize fresh material more effectively. The effect of the limestone was probably limited to reducing the soil acidity, which could also enable these bacteria to become more active. It was expected, therefore, that fresh carbonaceous material would be more rapidly decomposed in the presence of some of the released nitrogenous material. The reduction of acidity which gave rise to higher numbers of heterotrophic bacteria might also be assumed to stimulate the utilization of glucose.

DECOMPOSITION OF GLUCOSE

Samples from the plots at the two places had been allowed to dry rapidly in the air of the laboratory. The experiments began on July 13 (Soil T) and July 27 (Soil R), 1937. The treatment of the samples and the methods used have been described in a preceding paper (4).

The amounts of glucose recovered from the incubated samples are shown in Table III; the amount added was 32.1 mg. per 10 gm. of moist soil. After the glucose had disappeared, the pH value of the "nil" samples, determined colorimetrically, was 7.0 in both soils; that of the "CaCO₃" samples was from 7.5–8.0.

The carbon dioxide was collected at intervals for 10 days. The total amounts at the end of that time are given in Table III. Previous treatment with the limestone appears to have developed a more active microflora

in Soil *R*, for the increased evolution of carbon dioxide is seen in the control cultures as well as in those with glucose. The treatments do not appear to have been effective in the soil *(T)* more recently brought into cultivation, though activity in this soil was somewhat greater in the control cultures than in those of Soil *R*.

<div align="center">TABLE III</div>

Soil		Treatments			
		Nil	CaCO$_3$	Na$_2$CO$_3$	Na$_2$CO$_3$ + CaCO$_3$
		Glucose recovered, mg.			
R	At start	32.3			
	Day 1	24.9	26.8	24.6	22.9
	Day 2	8.0	Nil	5.5	1.8
	Day 3	Nil	Nil	Nil	Nil
T	At start	28.5			
	Day 1	23.2	17.0	19.8	18.6
	Day 2	Nil	Nil	Nil	Nil
	Day 3	–	–	–	–
		Total CO$_2$, mg. per 100 gm. soil			
R	With glucose	418	435	410	498
	Control	91	114	89	147
	Minus control	327	321	321	351
T	With glucose	483	492	456	483
	Control	161	188	139	156
	Minus control	322	304	317	327

The amounts of gas evolved from the glucose were about equal for all treatments, and were similar in both soils, except that that from "limestone" in Soil *T* was somewhat less, and that from "both amendments" in Soil *R* was increased; the differences may, however, be significant. The utilization of fresh energy material by a restricted microflora appears to have masked the differences brought about by the amendments.

The results suggest that the normal soil microflora had been stimulated by limestone, which effected a change in acidity only, and that any earlier benefits of the sodium carbonate, such as were reported previously (3) in studies of similar soils, were no longer in evidence.

The rates of carbon dioxide production were determined in the manner described in the previous paper (4); the results are given in Table IV, in which the values represent milligrams of carbon dioxide per 100 gm. of moist soil per hour.

The limestone appears to have remained more effective than the sodium carbonate; the latter stimulated activity only in Soil *T*, which contained

more of the residues remaining in a state of partial decomposition, and had developed higher numbers of bacteria. The earlier high rates in Soil *T* correspond with the earlier disappearance of the glucose. The numbers of

TABLE IV
CARBON DIOXIDE PRODUCTION, MG. PER 100 GM. SOIL PER HOUR

Soil	Time, hr.	Treatment			
		Nil	CaCO₃	Na₂CO₃	Na₂CO₃ + CaCO₃
R Cultures with glucose	23.25	0.86	2.46	2.20	3.40
	36.75	5.39	6.58	5.22	7.19
	45.25	6.72	8.47	6.66	6.03
	69.25	4.04	2.46	3.48	3.56
	143.75	1.28	1.12	0.95	1.29
	239.75	0.79	0.78	0.81	0.93
Control cultures	23.25	0.19	0.65	0.48	1.00
	36.75	1.18	1.73	1.13	2.13
	45.25	1.47	2.27	1.44	1.78
	69.75	0.88	0.64	0.75	1.05
	143.75	0.28	0.30	0.21	0.38
	239.75	0.17	0.20	0.17	0.27
T Cultures with glucose	23	—	4.54	3.48	4.53
	35	5.16	8.28	7.86	8.11
	44.5	—	6.35	6.75	7.40
	68.5	3.68	2.42	1.94	1.81
	140.5	1.52	1.34	1.31	1.31
	238.5	0.70	0.75	0.78	0.74
Control cultures	68.5	—	1.41	0.93	1.15
	238.5	0.68	0.54	0.44	0.46

TABLE V
BACTERIA AND ACTINOMYCES, MILLIONS PER GRAM

Time, days	Soil R				Soil T			
	Nil	CaCO₃	Na₂CO₃	CaCO₃ + Na₂CO₃	Nil	CaCO₃	Na₂CO₃	CaCO₃ + Na₂CO₃
	Cultures with glucose				Cultures with glucose			
At start	1.51	2.17	1.54	3.55	1.40	2.47	1.47	2.08
1	6.35	146.80	60.80	207.10	16.43	104.30	15.31	34.40
2	112.60	157.40	102.00	176.10	25.07	88.31	63.06	90.93
3	107.40	168.60	131.50	174.50	108.30	141.10	83.37	196.30
4	121.80	194.30	107.00	162.25	22.82	151.00	52.65	101.60
6	96.23	172.60	39.68	82.24	39.08	125.70	45.04	82.94
	Control cultures				Control cultures			
2	17.39	50.59	59.91	53.46	3.62	12.54	16.55	13.24
4	25.28	50.01	26.45	51.94	16.01	29.31	33.66	36.97

bacteria and actinomyces, in soil extract glucose agar, were as shown in Table V. The colonies in the samples treated with glucose were, after the start, bacterial only. The high numbers in the control cultures were caused by the long period of drying that preceded the remoistening of the samples.

The bacteria in the R cultures reached their highest numbers 24 hr. earlier than those in the T cultures, although the glucose was decomposed more rapidly in the latter. The limestone, both alone and with sodium carbonate, caused the greatest increases. In both soils the higher numbers with the limestone were associated with increased rates of carbon dioxide production from the samples receiving glucose.

The beneficial effects that may have arisen from the organic matter released by the sodium carbonate shortly after the soils had been treated were not shown as clearly as those derived from the treatment with limestone.

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VIBRIO AMYLOCELLA, N. SP., A SOIL ORGANISM THAT DECOMPOSES CELLULOSE AND PRODUCES GLUCOSE FROM STARCH[1]

By P. H. H. GRAY[2]

Abstract

Vibrio amylocella, n. sp., isolated from soil, attacks cellulose, starch, dextrin, and sugars. It does not grow in mediums containing beef extract. It can obtain its nitrogen from peptone, amino-acids, and mineral salt compounds, when supplied with a suitable source of carbon. Glucose is formed from starch and dextrin in liquid cultures, and accumulates in a starch medium containing ammonium chloride, but is utilized completely when potassium nitrate is supplied. The accumulation of glucose is related to the development of acidity, which renders the organism non-viable. Calcium carbonate depresses diastatic action and enables the organism to utilize the glucose formed.

The diastatic enzyme of *V. amylocella* is more active apart from the organism than in its presence. The organism is more active in starch hydrolysis than some common diastatic organisms.

Introduction

The decomposition of plant residues by soil bacteria and other fungi depends for completeness upon a great variety of micro-organisms, among which different groups play essential roles. Structural material such as cellulose, or storage products such as starch, are decomposed to yield ultimately carbon dioxide and water; the intermediate stages of the dissimilatory process in soil are not known but are assumed to be those found when such materials are acted on by micro-organisms under laboratory conditions. In such studies, cellulose and starch have given rise to sugars, fatty and other acids, acetone and alcohols. The bacteria described as the agents in that type of decomposition are mainly anaerobes, producing acids and alcohols from the sugars formed. Reference to such organisms and their activities are given by Thaysen and Galloway (16).

The production of sugars in soil has to be assumed, for they are so readily decomposed by aerobic as well as by anaerobic bacteria that they are not found as intermediate products in agricultural soil. Since, however, the sugars provide an easily utilizable source of carbon and energy, it would appear worth while to isolate and study specific micro-organisms that produce them from cellulose or starch. The present study concerns a bacterial organism that attacks cellulose and produces glucose from starch.

Among the bacteria that have been reported to produce glucose from starch are *B. suaveolens* (13), *B. anthracis* (10, 11), *B. maydis* (3), species of *Glycobacter* (18), *B. citreus cadaveris* and *B. cloacae* (8), *Granulobacter pectinovorum* (15), a coccus, not named (14), a cellulose-decomposing organism, not named (12), and *Streptobacterium plantarum* (4).

Glucose was shown to be present, by the osazone test, in the cultures of *Granulobacter*, of the cellulose-decomposing organism, and of the *Strepto-bacterium*. Napias (11) states that *B. anthracis* produces sugar whose nature she did not determine, but accepted as being partly glucose on the basis of work by Fernbach, who later reported maltose was produced by an extract of cells of *Tyrothrix (Bacillus) tenuis* (5); the maltose was changed to dextrose and then to dihydroxyacetone. In Napias' work the sugar was reported to have been destroyed before all of the starch had been hydrolyzed. Cavazzani (3) and Wollman (18) give figures for the yield of sugar (calculated as glucose); the former quotes a yield of 23 mg. of glucose from 100 mg. of starch in 48 hr. In Wollman's work, *Glycobacter proteolyticus* produced 2.5 gm. of sugar (calculated as glucose, but stated to be a mixture of glucose and maltose) from 6 gm. of starch. *Glycobacter peptolyticus* appears to have been less active.

The organism that forms the subject of the present study differs from any previously described species of the Schizomycetes in its cultural characters and biochemical activities. On the basis of its morphology, and from its ability to attack cellulose and starch in synthetic mediums, the binomial *Vibrio amylocella* is proposed for it.

Isolation and Cultural Characters

Vibrio amylocella, n. sp., was isolated in February 1938, from manured garden soil, of the brown-earth type over limestone. The sample contained a large amount of fibrous débris of grass roots. A suspension of the soil was inoculated into cellulose medium composed of a strip of filter paper partly immersed in 10 ml. of a mineral salt solution, with potassium nitrate 0.2%. The paper became disintegrated chiefly at the air-liquid interface. Actively motile bacteria of the curved rod form were isolated by means of an agar medium containing soluble starch. Colonies were light greenish yellow. Pure cultures decomposed filter paper cellulose and were strongly diastatic.

The organism was unable to grow in ordinary mediums containing beef-extract. Peptone could support growth when supplied as a source of nitrogen in starch hydrolysis, in the decomposition of cellulose, or with certain other carbonaceous compounds as sources of energy. Gelatin is slowly liquefied if a suitable source of carbon, such as starch, dextrin, or glucose, is present.

The organism, of which only one strain has been isolated, is aerobic; it does not utilize cellulose or starch in synthetic mediums from which atmospheric oxygen has been excluded. The young cells on starch agar are short, thin, curved rods, measuring about 2 to 3 μ long, or longer preceding division in the adolescent phase, by 0.6 μ broad. The motile cells from a culture on starch agar, after 24 hr. at 30 to 35° C., have from one to four polar flagella (Fig. 1). Capsules can be demonstrated by Muir's method, but are too tenuous to be seen in films prepared with nigrosin. In its morphology and manner of attacking cellulose, it thus resembles *Vibrio agarliquefaciens* (7), and some of the species of cellulose-decomposing vibrios studied by Kalnins (9). It differs from those organisms in that it does not grow in ordinary

mediums, nor liquefy agar. Among other mediums which did not support any growth were peptone broth, tryptophane broth, litmus milk, dextrose and lactose agars (both containing beef-extract), potato slant and block, potato dextrose agar, peptone glycerol broth, and glycerol, mannitol, inulin, and glycogen in synthetic mediums.
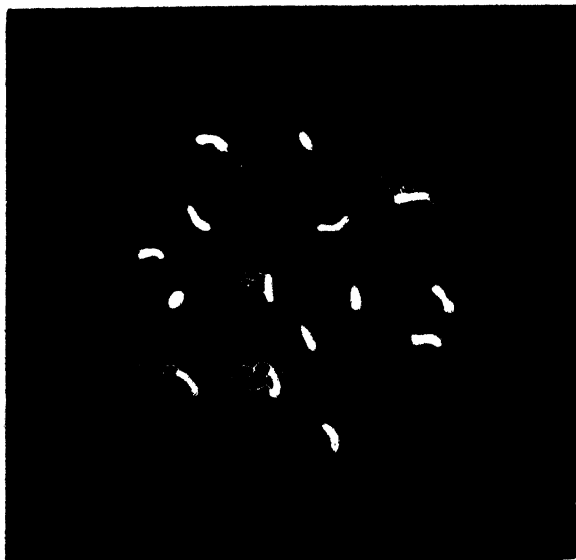
FIG. 1. *Vibrio amylocella, showing flagella, from a 24-hr. culture at 33° C. on starch agar. Stained by Gray's method (6). ×750. (Contact print from camera lucida drawing.)*

The action on cellulose is rapid; motile cells are visible in a wet mount from a culture after less than 24 hr. at 30° C., though disintegration of the paper may not then be visible. In some cultures, with potassium nitrate as the source of nitrogen, the paper begins to disintegrate within 24 hr. The organism decomposes the paper at temperatures from 15° C. to 37° C.; at the latter temperature the attack on the paper is more rapid than at 30° C. It does not grow at 41 to 42° C. Reducing substances have not been found in cellulose cultures. The amount of cellulose decomposed was ascertained by growing the organism in a mineral salts medium with potassium nitrate, with a round of filter paper weighing 0.55 gm. supported at the air-liquid level on glass beads in a 500-ml. Erlenmeyer flask; after one month the culture was filtered through a weighed paper; the loss in dry weight was 39%.

The organism develops catalase in a medium in which cellulose or dextrose has been attacked.

Methods

In the experiments, the results of which are reported below, the starch used, except where otherwise stated, was the Analar quality soluble starch from British Drug Houses, Limited (1). It was prepared for incorporation into

mineral salt or peptone mediums by dissolving cold water suspensions in boiling water. The suspension, in a small beaker, was poured a little at a time into the water, and the small beaker washed with more cold water. After boiling for 2 or 3 min., the opalescent liquid was mixed with the mineral salts or peptone solution, and the beaker washed with water into the mixture. With the higher percentages of starch, a small proportion of the starch adhered to the beaker, but the loss was probably negligible. Examination under the microscope showed that there were no whole grains of starch in the opalescent fluid.

The figures given below as "starch, per cent" added to a medium represent the amount of starch as weighed with the original moisture content, which was found to be 8% in the sample used. The values quoted for "glucose, mg. per 100 mg. of starch" do not allow for the small fraction of starch that did not enter the medium. The values include the allowance for moisture content. The results in terms of glucose can be accepted as approximate only, and are based on the ratio of dry weight of starch 91: glucose 100. Since loss of water by evaporation is a factor that has to be considered, at least in cultures that were incubated for the longer periods of time, some of the values reported may be too high. The results are presented, however, not as absolute values, but as comparative in any one set of cultures.

Reducing sugars were determined, in cultures filtered through a No. 3 Whatman paper, by Bertrand's method (2). By this method it was found possible to recover as much as 99.9% of a weighed amount of pure anhydrous glucose (dextrose) in solution, in repeated tests. The only modifications adopted in the method were to make the asbestos filter in a Gooch crucible, and to employ a Schuster bottle for small amounts of culture in the copper reduction process. The washings from the beaker, or bottle, as well as those from the filter, were tested with litmus paper until no trace of acid was present. Duplicate determinations with filtrates from cultures were in close agreement. In the results quoted in this paper, no allowance has been made for sterile control mediums, since it was found that the filtrates from these, after treatment with the reagents, changed colour with one drop of the permanganate solution.

Sources of Nitrogen

Suitable sources of inorganic nitrogen were found by inoculating mineral salt mediums, in 6 in. by ¾ in. tubes, containing nitrogen compounds as shown below, with carbon supplied as cellulose in the form of filter paper strips, or as 0.2% starch. The medium contained the mineral salts recommended by Thornton (17) for a standardized agar medium used in counting soil bacteria (Table I). The amount of nitrogen salt added was calculated to give 28 mg. of N per 100 ml. of medium.

In the starch cultures, after 48 hr., iodine gave a blue colour in Mediums 3 and 7; the brown-red colour of erythrodextrin was developed in the others.

After 72 hr. all of the starch and erythrodextrin had been destroyed in the mediums marked + +; erythrodextrin was still present in Medium 7, but later disappeared. Calcium nitrate did not support any growth with either source of carbon; the ammonium phosphate did not support growth in the cellulose medium. Nitrite was found in the cultures of both series supplied with potassium and sodium nitrate.

TABLE I

Medium	Source of nitrogen	Per cent	Growth with	
			cellulose	starch
1	KNO₃	0.200	+ +	+ +
2	NaNO₃	0.170	+ +	+ +
3	Ca(NO₃)₂	0.164	0	0
4	(NH₄)₂SO₄	0.132	+	+ +
5	NH₄NO₃	0.080	+	+ +
6	NH₄Cl	0.107	+	+ +
7	NH₄H₂PO₄	0.115*	0	+

* *Amount added in error for 0.230.*

Reducing sugar was found in the starch cultures having ammonium chloride; the phenylhydrazine test yielded glucosazone only.

The comparative values of different sources of nitrogen on diastatic action were determined by the plate method. A comparison was made between two different sources of mineral nitrogen. Plates of starch agar containing 0.1% potassium nitrate, and 0.2% ammonium chloride, were inoculated on the surface and incubated at 30° C.; the diameters of the colonies and of the diastatic zones were as shown in Table II.

Ammonium chloride was evidently preferred to potassium nitrate, though to a small extent and only after six days. This was confirmed in an experiment, reported below, in which the activity of this organism was compared with

TABLE II

Plate	KNO₃		NH₄Cl	
	Colony diam., mm.	Zone diam., mm.	Colony diam., mm.	Zone diam., mm.
	3 days			
a	1.5	20	3	23
b	3	27	5	23
c	4	27	5	28
	6 days			
d	3	30	3	41
e	3	34	3.5	39
f	3	35	4	40

that of others (Table X). The effect of different amounts of mineral-salt nitrogen was also tested. Plates were prepared with 10 ml. of agar with 0.2% starch, found to be the concentration giving the greatest activity in solution cultures, and ammonium chloride in the amounts shown in Table III. After three and six days duplicate plates were treated with iodine and the diameters of the clear zones measured.

TABLE III

EFFECT OF DIFFERENT AMOUNTS OF AMMONIUM CHLORIDE ON DIASTATIC ACTIVITY

NH₄Cl, %	Diameter of clear zone, mm.			
	3 days		6 days	
	Plate a	Plate b	Plate a	Plate b
0.025	22	23	36	37
0.05	18	20	35	32
0.10	19	18	38	34
0.20	18	18	37	36
0.40	22	22	38	40

There was no close agreement between the diameter of the colonies and the diastatic activity; thus, on the sixth day, with 0.2% ammonium chloride the colonies measured 5 mm. and 3 mm., while with one-fourth of that concentration the diameter of the colonies was 7 mm.; also, the colonies with 0.2% had not increased in diameter after the third day, though the diastatic zone had doubled in diameter. The concentration of ammonium chloride, within the range tested, had no clear-cut effect on size of colony or diastatic activity. It would appear that enzymic activity was not directly related to size of colony at the times the tests were made.

The effect of peptone, in comparison with ammonium chloride and potassium nitrate, is shown later, in Table X. Another test was made with glycine, *d*-alanine, and asparagine as sources of nitrogen, in comparison with ammonium chloride and with no added nitrogen, in starch agar plates and slant cultures. The amount of nitrogen supplied by each amino-acid was equivalent to that given by the salt, namely 52.8 mg. The diameters of the colonies and of the clear zones in the plates, after 5 days, were as shown in Table IV.

TABLE IV

Nitrogen source	Amount, %	Colony diameter, mm.		Zone diameter, mm.	
		a	b	a	b
Nil	—	1.0	1.0	16	13
NH₄Cl	0.200	2.5	3.0	29	27
Glycine	0.284	No growth		Trace only	
Alanine	0.300	1.5	2.3	17	21
Asparagine	0.250	3.0	—	27	—

The glycine apparently prevented growth, which was appreciable without added nitrogen.

The growth in the slanted agar containing starch was about equal in amount with asparagine and ammonium chloride, less with alanine, and only slight with no added nitrogen*.   At the same time, plates and slant cultures were prepared with these materials but without starch; there was some growth with the alanine and asparagine, but none with the glycine.

Viability; Effect of Calcium Carbonate

Early in this study it was noted that some cultures were no longer viable after about 10 days at 30° C.   This was found to be true of cultures containing ammonium chloride, but not of those containing potassium nitrate.   The former were found to develop acidity, while the latter remained neutral or became slightly alkaline.   A series of cultures was therefore prepared to determine how long the organism remained viable in a starch medium with ammonium chloride.   Cultures of 10 ml. in 6 in. by $\frac{3}{4}$ in. tubes were incubated at 20, 25, and 30° C.; the medium was composed of 0.2% starch, 0.1% ammonium chloride, inoculated in quadruplicate, one pair at each temperature having 0.1% calcium carbonate.   Transfers were made after 7, 9, 11, 14, 19, and 26 days into starch or cellulose mediums, by means of a triple-loop.   The number of days after which viable cells were still present was as follows:

At	20°	25°	30° C.
Without calcium carbonate	26+	14	11
With calcium carbonate	26+	26+	26+

The test was not continued after the 26th day.

The effect of calcium carbonate was also tested in plate cultures; the medium was the same as that used in the test for viability, with the addition of 1.0% agar.   The plates were treated with iodine and the clear zones found to measure as shown below; each value represents the mean diameter of the clear zone of two cultures, in millimeters:

	4 days	7 days
Without calcium carbonate	34	44
With calcium carbonate	29	34

Diastatic activity was thus depressed in the presence of calcium carbonate. It was also found that, in addition to prolonging viability, calcium carbonate enabled the organism to utilize the glucose formed.   The cultures grown at 20° C., in the test for viability, were later used for a determination of reducing power.   In the culture without calcium carbonate, a large amount of cuprous oxide was obtained in the reduction test on the 35th day; none was obtained from the culture with calcium carbonate.

The organism did not grow in solution cultures containing starch without added nitrogen.

Effect of Potassium Nitrate

As alkaline conditions enabled the organism to utilize the glucose that accumulated in mediums with ammonium chloride but without calcium carbonate, a test was made to determine the fate of the glucose in cultures containing 0.2% starch and 0.1% potassium nitrate, because the utilization of this salt rendered the medium alkaline. The cultures were grown in 50-ml. quantities in Erlenmeyer flasks of 125 ml. capacity. Tests were made, at the times shown in Table V, for disappearance of starch and for the amount of sugar formed, calculated as glucose; the total numbers of bacteria were counted by means of a haemocytometer.

TABLE V

DECOMPOSITION OF STARCH, WITH NITROGEN AS POTASSIUM NITRATE

Hours or days	Iodine test	Glucose, mg. per 100 mg. starch	Bacteria, millions per ml.
12 hr.	Starch	4.2	134
47 hr.	Starch	6.0	200
63 hr.	Erythrodextrin	3.6	420
70 hr.	Nil	12.9	800
86 hr.	—	27.8	880
7 days	—	5.4	2400
12 days	—	Nil	—

It is evident that with potassium nitrate as the source of nitrogen the glucose formed was utilized by the increased numbers of bacteria after the 86th hr. It would also appear probable, from a comparison with results obtained in tests with glucose, reported below, that glucose was being used to some extent during the earlier stages.

Although the growth of the organism proceeded while glucose was beginning to accumulate, it is not reasonable to conclude that energy was being derived from the hydrolysis. From the test with potassium nitrate as the source of nitrogen, it seems conclusive that the sugar was the source of energy after the 86th hr. With ammonium chloride supplying nitrogen, the accumulation of the sugar may be due to the progressive dissolution of the cells in an acid medium.

Evolution of Carbon Dioxide

An experiment was made to ascertain when the metabolic activity of the organism was at its highest in cultures containing ammonium chloride. Flasks of 500 ml. capacity, containing 200 ml. of 0.2% starch and 0.2% ammonium chloride, were inoculated and connected to tubes of baryta for the absorption of carbon dioxide by aspiration of air previously freed of that gas. Two tubes, each with 20 ml. of 0.105 N barium hydroxide, were used in series to collect the carbon dioxide from each culture. The whole apparatus was placed in an incubator at 28 to 30° C. The carbon dioxide was determined at intervals as shown in Table VI.

TABLE VI

Hours or days	Carbon dioxide, mg.		Glucose, mg. per 200 ml.
	Amount	Per hour	
25 hr.	69.6	2.78	
30 hr.	20.2	4.05	
35.25 hr.	11.9	2.27	
48.25 hr.	14.3	1.12	24
108 hr.	12.8	0.21	—
5 days	—	—	48
13 days	—	—	89
Total in 4.5 days	128.8		

The amount passing over into the second tube is included in the value quoted for the 25th hr.; the amount collected at the 108th hr. in the second tube was only 5.7 mg., which has been omitted from the total.

The organism evidently developed its maximum metabolic activity before the 48th hr., when there was no trace of starch or erythrodextrin. The amount of carbon dioxide collected in 48 hr. was equivalent to 79 mg. of glucose oxidized. The pH of the medium was 5.2 at the 108th hr.

The fact that sugar was not found until metabolic activity had slowed down would suggest that, under these conditions, either (i) the organism obtained its energy by the hydrolysis, and the production of glucose was due to the release of the enzyme dextrinase from dying or dead cells, or (ii) some glucose was produced during the period of greater activity in an amount sufficient to supply energy (by oxidation) to the extent shown, the hydrolytic enzyme being restricted by the aeration, so that the glucose accumulated to an extent relatively less than it did in non-aerated cultures incubated for the same length of time. The former alternative would seem unlikely, since the possession of an enzyme that leads to the production of energy material from a substrate pre-supposes that the organism will use all or part of the energy so produced; the accumulation (or non-utilization) of part of the energy source set free by the enzyme may, nevertheless, be due to the cause stated. In regard to the latter alternative, this would appear to receive support from the fact that the organism utilizes glucose in the presence of ammonium chloride, as shown below in the section headed "Other sources of carbon".

Concentration of Starch

The action of the organism on different concentrations of starch was tested with plate cultures and with solution cultures. Plate cultures were prepared with starch in the concentrations shown in Table VII and with 0.2% ammonium chloride added to mineral salts agar. The relative concentrations of starch and nitrogen were the same as those used in the test in which different concentrations of ammonium chloride were used. The plates were incubated at 28° C., and tested with iodine on the third and sixth days.

TABLE VII

DIASTATIC ACTION OF *V. amylocella* IN AGAR WITH DIFFERENT CONCENTRATIONS
OF STARCH

Starch, %	Diameter of clear zone, mm.			
	3 days		6 days	
	a	b	a	b
0.1	20	—	36	42
0.2	21	—	40	43
0.4	18	17	33	38
0.8	15	14	28	27
1.6	8	8	25	—

At the same time, cultures on peptone agar with 1.0% starch were prepared; the clear zones measured 7 to 8 mm. on the third day and 20 to 21 mm. on the sixth.

It would appear that, in the earlier stages, a concentration of starch over 0.2% depressed diastatic action; on the sixth day, however, although the lower concentrations developed the greater areas of diastatic activity, the relative increase over that shown at the third day was greatest in the culture having the highest concentration. The mineral nitrogen was preferred to the peptone, the concentration of which was 0.5%.

Two series of cultures were made for testing the activity in solution cultures. In both series, different concentrations of starch were used in Erlenmeyer flasks of 125 ml. capacity, containing 50 ml. of medium; nitrogen was supplied as ammonium chloride. The cultures were incubated at 28 to 30° C. In the first series, portions were removed aseptically at intervals for the determination of reducing sugar (glucose). The results shown in Table VIII include three only of the concentrations used.

TABLE VIII

AMOUNTS OF GLUCOSE FORMED FROM DIFFERENT
CONCENTRATIONS OF STARCH

Hours or days	Glucose, mg. per 100 mg. starch		
	0.4% starch	0.8% starch	1.2% starch
60 hr.	25	23	14
90 hr.	78	51	54
110 hr.	73	48	43
6 days	—	—	56
8 days	82	69	70
12 days	—	73	—

The experiment showed that the higher concentrations of starch reduced the diastatic activity in the early stages. This was further confirmed in the second series of cultures, which were supplied with starch, 0.2% and 1.0%.

Eight flasks of each medium, having a pH value of 7.2, were inoculated with one drop each of a suspension of the organism. At intervals of five days, duplicate cultures were taken for the determination of the pH value by the colorimetric method, and for glucose; the results are shown in Table IX.

TABLE IX

Culture	0.2% starch				0.1% starch			
	pH		Glucose*		pH		Glucose*	
	a	b	a	b	a	b	a	b
Day 5	5.7	5.7	49	44	5.7	5.7	22†	25†
10	5.8	5.7	52	52	5.5	5.7	53	58
15	5.5	5.5	52	56	5.2	5.3	60	62
21	5.6	–	54	–	5.2	–	63	–

    * *Mg. per 100 mg. of starch supplied.*
    † *A trace of erythrodextrin was found.*

The cultures were not viable on the 10th day, after which there was little change in the pH values or the amounts of glucose. The phenylhydrazine test with these cultures yielded only glucosazone.

Action of the Enzyme

The action of the enzyme in the absence of proliferating or viable bacteria was tested in plate cultures and in solution cultures. Plate cultures of starch agar were incubated for two days, when the diastatic zone had developed sufficient area to permit blocks of agar to be cut and transferred to the surface of sterile agar, or into sterile liquid medium. Tests with iodine in parallel plate cultures, two days old, showed that the diastatic zone from which the blocks were cut contained no starch. The results of an experiment to compare the enzyme action with that of a colony are shown below. The plates with the blocks were incubated for three days.

Size of blocks cut at 2 days, mm.	Enzymic zone diameters, mm.	
	Plates with blocks, 3 days	Original plates, 5 days
3 by 4	37	36
8 by 10	42	44

It should be noted that the clear zones produced by the blocks had about the same diameters as those of the corresponding colonies, which were incubated for two days longer than the blocks. This may be interpreted as relatively greater activity of the enzyme both from its shorter period of contact with the starch, and from the fact that the blocks represented only a small percentage of the enzymic zone from which they were cut.

Blocks cut from enzymic zones in agar were also transferred aseptically to 10-ml. portions of liquid medium containing 1.0% starch and 0.2% ammonium chloride. Two large blocks (6 by 7 mm., and 7 by 8 mm.) and two small blocks (3 by 3 mm.) were each placed in a tube of medium. After 48 hr. at 30° C., there was no starch in the tubes having the larger blocks; after 72 hr., erythrodextrin was present in the medium containing the small blocks; this had disappeared on the 5th day. On the 13th day, the medium containing the largest block was found to contain about 40 mg. of glucose (allowance being made for evaporation of water). It would appear, therefore, that the enzyme developed by a portion of a colony may produce a considerable amount of glucose from soluble starch. Reducing sugar was not, however, found in plates of starch agar in which the organism, or its enzyme, had hydrolyzed the starch.

The action of the enzyme in the absence of proliferating bacteria was also tested in 10-ml. solution cultures having 1.0% starch and 0.5% ammonium chloride. After two days of growth at 30° C., starch was still present; some cultures were then treated with toluol, and others allowed to continue growth. After 24 hr. there were no viable cells in the tubes treated with toluol, while actively motile bacteria were seen in the control (untreated) cultures; erythrodextrin was present in both series. On the 5th day the erythrodextrin had disappeared, and on the 10th day the bacteria were not viable in the control cultures. The amount of glucose, in milligrams per 100 mg. of starch supplied, was determined at intervals; the results are shown below:

Days	Treated	Control
3	14	10
7	33	22
20	54	29

These results may be interpreted in two ways; the enzyme may be more efficient in the absence of living organisms, or the organism decomposed part of the glucose formed. As shown below, the organism is able to decompose a large amount of glucose in cultures having ammonium chloride as the source of nitrogen and glucose as the source of carbon.

Other Forms of Starch

Potato starch (Baker and Adamson quality, from the General Chemical Co., New York) serves as well as soluble starch as a source of carbon in a mineral salts medium. A 0.2-gm. portion of the starch was dissolved, as described previously, and mixed into 100 ml. of a medium with 0.1% ammonium chloride. Growth was visible, and motile cells were seen, within 24 hr. On the third day there was no trace of the colour of erythrodextrin by the iodine test. On the seventh day the cultures were acid, requiring 3.5 ml. of 0.1 N sodium hydroxide per 100 ml. to be neutralized. The amount of cuprous oxide obtained was equivalent to 57 to 58 mg. of glucose per 100 ml. of medium; (this value has not been adjusted to account for moisture or on

the basis of starch 91 : glucose 100). The phenylhydrazine test yielded crystals of glucosazone only. The yield of sugar from corn-starch (Kahlbaum) was 47 mg. in 10 days.

Comparison with Other Micro-organisms

Bacillus ruminatus, a common soil organism active in starch hydrolysis, and *Aspergillus oryzae* were compared with *V. amylocella* in plate cultures of starch agar. The organisms were each inoculated onto the surface of four plates of 0.2% starch agar, with nitrogen as 0.1% peptone, 0.1% potassium nitrate, and 0.2% ammonium chloride, and incubated at 30° C. After three and six days, duplicate cultures of each were treated with iodine, and the diameters of colonies and enzymic zones were measured.

The results are given in Table X, in which the figures represent the mean diameters of colonies and zones of complete hydrolysis (as shown by a clear white area) in two plates on each day, and also the ratios that the diameters of the colonies bore to the diameters of the clear zones. It should be noted

TABLE X

COMPARISON BETWEEN *Vibrio amylocella, Bacillus ruminatus,* AND *Aspergillus oryzae*
IN PLATES OF STARCH AGAR

Diameters of colonies and enzymic zones in millimeters, and their ratios

Organism		Peptone		KNO$_3$		NH$_4$Cl	
		Colony	Zone	Colony	Zone	Colony	Zone
		Cultures 3 days old					
V. amylocella	Diam.	0.8	11.5	1.5	16.0	1.5	21.0
	Ratio	1	15.32	1	10.67	1	14.00
B. ruminatus	Diam.	4.0	7.0	3.0	5.0	2.5	4.0
	Ratio	1	1.75	1	1.67	1	1.60
A. oryzae	Diam.	23.0	24.0	9.0	10.0	20.0	21.0
	Ratio	1	1.00	1	1.01	1	1.00
		Cultures 6 days old					
V. amylocella	Diam.	2.0	16.0	2.25	15.0	2.25	31.0
	Ratio	1	8.0	1	6.8	1	13.8
B. ruminatus	Diam.	6.0	16.0	4.5	7.0	3.0	5.0
	Ratio	1	2.67	1	1.23	1	1.67
A. oryzae	Diam.	48.0	56.0	21.0	25.0	38.0	40.0
	Ratio	1	1.17	1	1.19	1	1.05

here that the actual diameters of zones of partial hydrolysis by *B. ruminatus* and *A. oryzae* were larger than those quoted, but the comparison was made with the complete hydrolysis effected by *V. amylocella*. *B. ruminatus* had three narrow zones: an inner white zone, a middle zone tinged with blue,

and an outer purple zone indicating the presence of erythrodextrin. *A. oryzae* developed also a blue-tinged wide border of incomplete hydrolysis. The ratio of colony to these outer enzymic zones was still smaller than that shown by *V. amylocella*, at the third day the ratios being, for *B. ruminatus* 1 : 4.33 (inner and outer zones together) and for *A. oryzae* 1 : 1.6.

The results from the plate cultures show that *V. amylocella* is considerably the most active organism, in proportion to the amount of colonial growth.

Cultures of the bacteria, including *B. dendroides*, *B. mesentericus*, and *B. mycoides*, were also grown in liquid medium containing 1.0% starch and 0.5% peptone. *B. dendroides* was used in this test, since it is very active in starch hydrolysis. This organism is not so useful for comparison in plate cultures, because of its habit of spreading irregularly in the water film on the surface of agar plates; the width of the enzymic zone from the edge of the growth was about 2 mm. only. In the liquid cultures, *V. amylocella* decomposed the starch in 3 days; *B. dendroides* in 11 days. After 17 days none of the other spore-formers tested had destroyed the starch completely; erythrodextrin was formed only by *B. ruminatus*.

Other Sources of Carbon

As the conversion of starch to glucose by this organism passed through a stage in which iodine gave the colour of erythrodextrin, it was assumed that colourless dextrin would also be a product of the hydrolysis. A medium containing 1% dextrin (Kahlbaum) and 0.1% ammonium chloride was prepared in 100-ml. quantities in Erlenmeyer flasks of 500 ml. capacity. After sterilization, a test was made for reducing value; 10 ml. gave a value equivalent to 4.4 mg. glucose per 100 mg. of dextrin. Eight days after inoculation, glucose was found to be present by the osazone test; the amount in 20 ml. of filtered culture was equivalent to 33 mg. per 100 mg. of the dextrin supplied; the same value was found five days later, by which time the culture was no longer viable.

The action of the organism on glucose (dextrose) was tested with two sources of nitrogen, as it was found that with potassium nitrate the sugar produced from starch was utilized, while with ammonium chloride it accumulated. This was also found to be partly true with dextrose as the sole source of energy. The organism was incubated at 30° C. in flasks containing 20 ml. of mineral salt medium with dextrose (B.D.H. "Analar" quality) in the concentration nearly equivalent to the amount available from 0.2 gm. of starch, *i.e.*, 0.2%, and with 0.2% ammonium chloride. The amount of dextrose recovered, in duplicate tests with the sterile medium, was 95% of the amount added. After two days the amounts of dextrose decomposed were 66% in the potassium nitrate culture and 19% in the ammonium chloride culture. The pH values of the two cultures were 7.2 and 6.0 respectively. On the fifth day all of the sugar had been utilized in the potassium nitrate culture; 57% had been utilized in the ammonium chloride culture, in which the pH value was 5.7. Catalase was present in the culture with potassium

nitrate, the amount of gas produced in the closed arm of a fermentation tube being 33% of the volume of liquid; there was none in the culture with ammonium chloride.

Oxalic acid has been found as a product of the attack on glucose. The organism was inoculated onto plates of glucose agar containing potassium nitrate and calcium carbonate; after a few days octahedral crystals identical with those of calcium oxalate were produced abundantly around the colonies.

Other sources of carbon were also used in synthetic mediums. Lactose, maltose, sucrose, galactose, and raffinose all supplied sources of energy. Sucrose was inverted, the presence of fructose being ascertained by means of Seliwanoff's reagent; typical osazone crystals were also found. A medium with 1% sucrose yielded invert sugar equivalent to 54% of the original sucrose, in eight days; the yield increased to 59% on the 12th day. A medium with 2% sucrose, after 60 days of incubation, yielded invert sugar equivalent to 95% of the original sucrose.

Glucose and fructose were formed from raffinose, but the yield of copper was not more than 9.3 mg. per 100 mg. of the trisaccharide; in a six-days-old culture the osazone crystals resembled those found in the sucrose culture, but crystals of galactosazone could not be found.

In a medium containing 0.2% galactose and 0.1% ammonium chloride, the yield of cuprous oxide on the seventh day was equivalent to 45 to 50 mg. of reducing sugar, calculated as galactose, per 100 mg. of galactose supplied. The amount had not increased on the 10th day. The phenylhydrazine test yielded crystals of galactosazone and brown globular masses of short, blunt-ended crystals that have not been identified. In a medium with 1.0% galactose, the organism utilized only 14% of the sugar in 18 days. The osazone test gave the same results as in the previous test.

Lactose and maltose were decomposed without yielding any trace of glucose; nor did the culture with lactose yield galactose. In 12 days, with ammonium chloride, only 16% of the lactose and 13% of the maltose were utilized. On the other hand, when potassium nitrate replaced the ammonium salt, all of the sugar in a 0.2% maltose medium was decomposed in eight days.

The organism thus appears to be able to decompose these carbohydrates more energetically in the lower concentrations, and when supplied with potassium nitrate in place of ammonium chloride.

The organism grew poorly in a medium containing 0.2% trehalose, and did not produce any glucose.

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MICROBIC DISSOCIATION OF LACTIC ACID STREPTOCOCCI[1]

BY OLGA OKULITCH[2]

Abstract

The induction of microbic dissociation of the lactic acid streptococci by the presence in the medium of a carbohydrate with a specific configuration has been studied.

Strains of *Streptococcus lactis* and *Streptococcus cremoris* have been shown to undergo dissociative changes when grown in the presence of glucose. Lithium chloride and phenol have been found to be without influence. The change from the *S*- to *R*-form was accompanied by loss of lactose-fermenting ability.

The cultural, colonial, and biochemical characters of the organisms at different stages of variation have been described.

It is suggested that the organism must be in a susceptible condition before dissociation can be induced.

In a preliminary note (11), the inhibitory power of specific carbohydrates on the ability of lactic acid streptococci to form acid from lactose in milk or in broth was described. It was suggested that this restraining influence on acid formation was related to the stereoisomeric structure of the hexoses. Although no reference was made at the time to the influence of the sugars on the morphological and cultural appearance of the organism, distinct changes in these characters, which seemed to be connected in some way with the loss of lactose-fermenting ability, were observed. A review of the literature on the question of bacterial dissociation suggested that the cultural and colonial variations which we had encountered were of the same nature as those described by Hadley (5, 6). As the work progressed, it appeared probable that we were dealing with a phenomenon of dissociation in the lactic acid streptococci and that the microbic dissociation had been induced by the presence in the medium of a carbohydrate having a specific configuration.

The work reported upon herein is a detailed study of the bacterial dissociation of strains of lactic acid streptococci grown in media containing the carbohydrate, glucose. In an attempt to establish the sequence of variation in cultural and colonial characters, several strains of *Strep. cremoris* and *Strep. lactis* have been employed. In addition to the *Strep. cremoris* strain No. 142, described in a previous paper (11), the following organisms were selected for intensive study: *Strep. cremoris* 114, *Strep. lactis* 146, and *Strep. lactis* 232 (10). Certain observations on microbic dissociation have also been carried out in the case of several other streptococcus cultures.

Experimental

The induction of dissociation and the accompanying inhibition of lactose-fermenting ability were brought about by serial cultivation of a vigorous culture of the strain under investigation in casein digest broth (4) and in peptonized milk broth (4), the broth in each case containing 2% glucose. Serial transfers in each broth were made at 48-hr. intervals. At every transfer, milk also was inoculated with the culture from the sugar broths, and the time required to clot the respective milk tubes determined. At frequent intervals poured plates on Difco peptonized milk gelatin were made from the broth cultures. The colonies were examined with a low power microscope, giving a ×40 magnification.

Cultural Characters   ## Discussion

Dissociation among lactic acid streptococci results in the development of a variety of colonial types. The commonest initial change is from a round, smooth colony (Fig. 1) to a slightly lobate form (Fig. 2). If these lobate colonies are allowed to age for one to two weeks, outbursts consisting of a very fine rough growth from one or more points in the margin of the colony are formed (Fig. 3). In certain instances, aging of the lobate colonies leads to the formation of a fringe of daughter colonies, or papillae, around the entire colony (Fig. 4). At the time that these changes in the colony form appear it is possible that the organism is in the transition stage from the *S*- to the *R*-type. The picking of these intermediate-type colonies never gives a broth-culture characteristic of a pure smooth or rough strain. On plating this culture, a mixture of smooth, lobate, and pure rough colonies is obtained. A pure rough culture of a lactic acid streptococcus plated on peptonized milk gelatin usually gives rise to a rough, filamentous, spider-like colony (Fig. 6). With certain strains, however, a more compact, although definitely filamentous type of colony, is to be seen (Fig. 5).

On several occasions, in addition to the characteristic *S*- and/or *R*-types of colonies which appeared on peptonized milk gelatin plates, very small pin-point colonies were also to be seen. On inoculation of these colonies into broth, a cloudy growth resulted, which, on transfer, failed to produce acid in milk. On replating, the culture reverted to the normal *S*-form, and evidenced the cultural characters of the original strain, including the ability to form acid from lactose in milk. Colonies of this type have been described by Dutton (3) and also by Staryghina (13).

The morphological appearance of the pure *S*-form of lactic acid streptococci is characterized by a regularity in the size and shape of the cells, which occur usually in pairs or short chains (Fig. 7). On dissociating to the stable *R*-form, the culture becomes decidedly long-chained, the cells at times being very irregular in shape (Fig. 8).

The change in morphology is always accompanied by a characteristic change in type of growth in liquid media. It has been pointed out by other workers in the field of microbic dissociation that occurrence of agglutination

in broth is associated with the rough type of colony (1, 3, 6, 12, 13, 14). All
the organisms studied produced a homogeneous cloudy growth in broth when
in the *S*-form, and a mucoid, gelatinous growth suspended in the clear super-
natant medium when in the *R*-form.
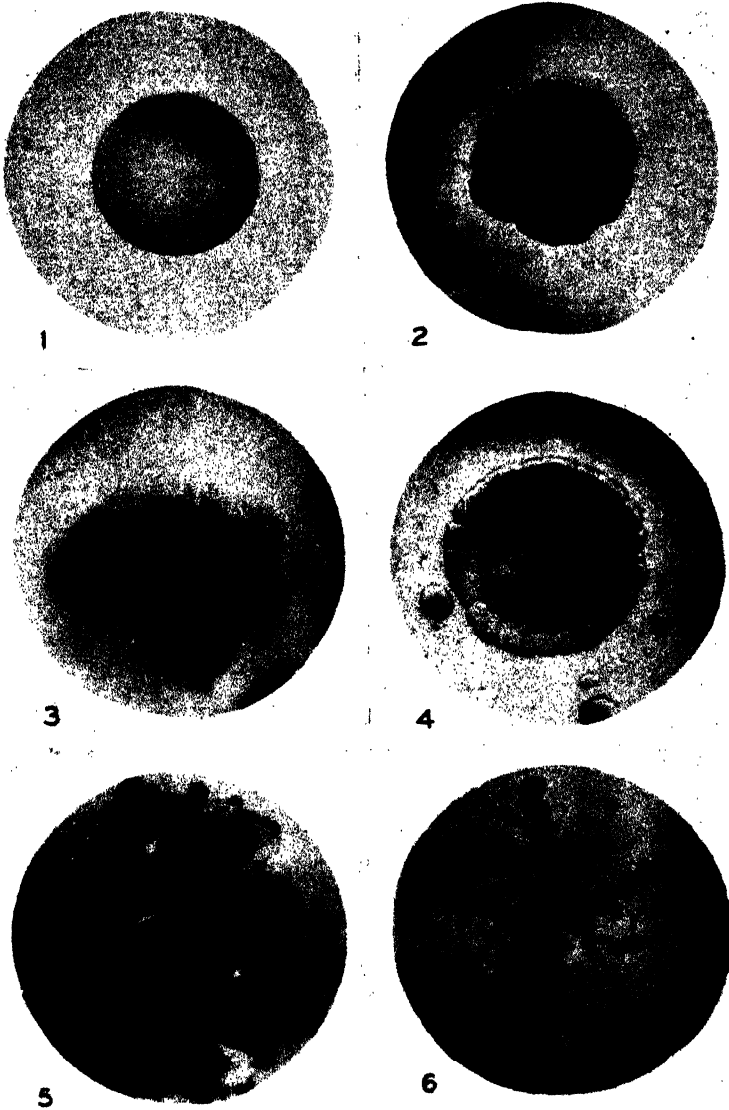
FIGS. 1–6. *All colonies were grown on peptonized milk gelatin.* ×35. FIG. 1. *Strep.
cremoris 114, smooth colony.* FIG. 2. *Strep. cremoris 114, lobate colony.* FIG. 3. *Strep.
cremoris 114, aged colony showing outbursts.* FIG. 4. *Strep. cremoris 114, aged colony showing
formation of daughter colonies.* FIG. 5. *Strep. cremoris 114. rough colony.* FIG. 6. *Strep.
cremoris 142, rough colony.*

A difference in the type of growth may also be observed in agar stab cultures.   A pure smooth strain produces an even filiform growth along the line of inoculation (Fig. 9a).   At some stages of growth, outbursts from several points in the stab are formed (Fig. 9b).   These outbursts are probably the same type of transition growth that is formed in colonies aging on gelatin plates.   The true *R*-culture gives a very fuzzy arborescent growth in agar stab culture.   The majority of strains studied seem to be more aerobic in this condition than in the *S*-form, the growth terminating at a point approximately half-way down the stab (Fig. 9c).   The exception to this is *Strep. cremoris* 114. This organism, when in the rough stage, usually grows only at the bottom of the tube (Fig. 9d).
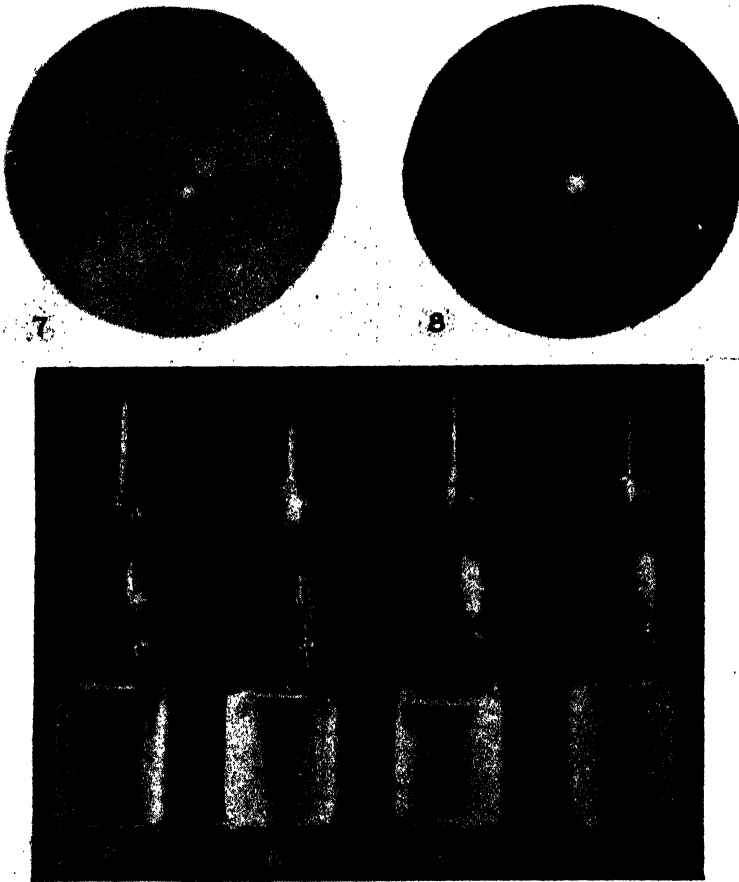
FIGS. 7–9.   FIG. 7. *Strep. cremoris 142, smooth strain; 48-hr. broth culture. Gram stain.* ×1100.   FIG. 8. *Strep. cremoris 142, rough strain; 48-hr. broth culture. Gram stain.* ×1100. FIG. 9. *Three-week-old agar stab cultures.   (a) Strep. lactis 232, smooth strain.   (b) Strep. lactis 232, agar stab showing outbursts.   (c) Strep. lactis 232, rough strain.   (d) Strep. cremoris 114, rough strain.*

Biochemical Characters

The formation of a true *R*-dissociant results in the complete loss by the organism of the ability to ferment lactose, either in milk or in broth. This biochemical variation sometimes occurs prior to the onset of dissociation as revealed by colonial and cultural characters. When this phenomenon occurred, the cultural and colonial changes associated with dissociation usually developed in the course of a few transfers. Although some evidence has been obtained that there is a tendency for the *R*-form to revert to the colonial characters of the *S*-form, a complete reversion has not as yet been accomplished, and on no occasion has a rough form ever acquired lactose-fermenting ability. In confirmation of previous findings (11), the inhibition of lactose-fermenting ability is not accompanied by a failure to ferment the monosaccharide glucose. The *R*-variant continues to ferment glucose with a vigour equal to that possessed by the *S*-form.

The induction of dissociation and the accompanying inhibition of lactose-fermenting ability took place most readily in peptonized milk broth containing 2% glucose. The addition of 0.25% lithium chloride or of 0.1% phenol to either peptonized milk broth or casein digest broth, failed to induce dissociation. A similar finding in the case of *Streptobacterium plantarum* has been described by Tracy (15). In contradiction of the results obtained by Tracy and those reported upon herein, Staryghina (13) was able to induce microbic dissociation in the case of both lactic acid streptococci and streptobacteria by transfer in lithium chloride or phenol broths.

SUSCEPTIBILITY TO DISSOCIATION

The number of transfers required to bring about microbic dissociation varied not only for different streptococci, but also for the same culture grown under similar conditions on different occasions. This inconsistency in the behaviour of the lactic acid streptococci, which has also been described by Whitehead and Cox (16), was especially marked in the case of *Strep. lactis* 146.

At first this organism dissociated very readily, rough colonies making their appearance at approximately the twentieth transfer. The rough culture continued to show abundant growth in the medium for four subsequent transfers. The fifth glucose peptonized milk broth tube, however, remained apparently sterile. No colonies developed on poured plates made from this broth tube.

Based on the assumption that the sudden cessation of obvious growth following closely upon a change from the *S*- to *R*-type was analogous to the development of a *G*-form as described by Hadley, Delves and Klimek (7), Duff (2), Kopeloff (9) and others, in several different types of micro-organisms, the Hauduroy (8) "washed plate" technique was applied to the apparently sterile broth tube in an attempt to demonstrate the existence of this form of the lactic acid streptococci. Although the washed plate series was carried to the sixth plating, no growth was observed, and consequently the series was discontinued.

Repetition of the work described above gave similar results as far as the sequence of dissociative changes was concerned, but the change from the *S*- to the *R*-type, with subsequent failure to grow, took place much more rapidly.

The phenomenon of dissociation was not encountered on repetition of this experiment at a later date. Even after 60 transfers in glucose peptonized milk broth, followed by 15 serial transfers in casein digest broth to which 0.1% phenol had been added, no obvious change in any of the characters of the organism was observed. The colony form remained round and smooth, the growth in broth was always cloudy, and there was no evidence of loss of lactose-fermenting ability.

Of the cultures studied, *Strep. lactis* 146 was the only strain found to exhibit the phenomenon of sudden cessation of growth on serial transfers of the *R*-forms in broth. In all other strains, a stable *R*-form appeared on dissociation.

It is evident that the phenomenon of dissociation in the lactic acid streptococci is erratic in its appearance, and it would appear to depend on the sensitivity of the organism to the experimental conditions employed for the induction of dissociation. Microbic dissociation will not occur unless the micro-organism is in a susceptible condition. It has not been possible as yet to determine the factors responsible for the change of an organism from a stable smooth form to a smooth form susceptible to variation.

Conclusions

The results of the work reported upon herein clearly show that microbic dissociation of lactic acid streptococci may be induced by the presence in the medium of a carbohydrate with a specific configuration. It is suggested that the micro-organism must be in a susceptible condition before dissociation can occur. The variations in cultural and colonial characters are not constant for different strains of the same species, nor does the same strain follow a definite sequence of changes on dissociation at different times. The variations that occur among the lactic acid streptococci on dissociation are similar to those described for other bacterial species.

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VEGETATIVE PROPAGATION OF CONIFERS

I. ROOTING OF CUTTINGS TAKEN FROM THE UPPER AND LOWER REGIONS OF A NORWAY SPRUCE TREE[1]

BY N. H. GRACE[2]

Abstract

Cuttings from the upper and lower regions of a Norway spruce tree were treated with talc only, and with talc containing 1000 p.p.m. indolylacetic acid. Ten weeks after being planted in sand, 43% of the upper and 75% of the lower cuttings were rooted. Hormone treatment increased the number of roots per rooted cutting but decreased the mean length of root. Lower cuttings produced twice the length of root of the upper cuttings, and the mean length of individual roots was also significantly greater. Nineteen weeks after being planted, the cuttings not rooted at 10 weeks were re-inspected, and gave final rooting values for the experiment of 48% for upper cuttings and 86% for lower. Physiological differences are consequently suggested in cuttings taken from the upper and lower regions.

Vegetative propagation of conifers is essential to rapid improvement through forest tree breeding, because valuable parental stock and hybrids could be multiplied and tested by this means; but comparatively little is known concerning the factors affecting successful propagation of cuttings from many varieties of tree. The position of the cutting on the tree is one of the factors to be considered. It is possible that there are physiological differences in the rooting response of cuttings taken from the upper and lower regions of the spruce, since the upper region bears the female flowers, the lower the male, and the wide-spreading lower branches of some varieties have a tendency to layer. Accordingly, a small preliminary experiment now to be described was carried out in the greenhouse of the National Research Laboratories in order to investigate this point.

Experimental

In mid-November 1938, branches were collected from a typical Norway spruce (*Picea excelsa*) tree, approximately 18 years of age and situated in a plantation at the Dominion Forest Station, Chalk River, Ontario. The branches, taken from what were roughly the upper and lower third portions of the tree, carried current year's growth of approximately the same length. The bases of the branches were packed in moist peat, and the package left outside in this manner until mid-January 1939. The dormant material was then divided into full-length cuttings, which were torn off at the node; jagged edges were trimmed with a knife, below the heel. There were 90 cuttings from each position, comprising terminals and laterals in the approximate ratio of one terminal to four laterals. Cuttings ranged from 2 to 4 in. in

length, and were divided into groups of 15, each group being representative of the different lengths. Prior to planting, three replicates of 15 cuttings from each position were dusted with talc only, and three with talc containing 1000 p.p.m. of indolylacetic acid. Random arrangement in the propagation frame permitted statistical analysis of the results for differences due either to position or to chemical treatment.

The cuttings were planted in washed brown sand in a cotton-covered propagation frame placed in the greenhouse and equipped with electrical bottom heat cables. The room temperature ranged around 65° F., while the sand temperature was held at approximately 72° F. The cuttings were held in this frame for 10 weeks, when they were dug up for observation; the unrooted cuttings were then replaced in the frame for a further period of 9 weeks, when final observation was made.

Results

Record was made of the number of cuttings rooted in each replicate of 15; further, the number of roots and their length were determined and expressed as number of roots and millimetres of root per rooted cutting. Finally, the mean root length was calculated. These four sets of data were subjected to analyses of variance to bring out the effects of position of the cutting on the tree, treatment with 1000 p.p.m. indolylacetic acid, and the interaction between position and treatment.

The results of the four mathematical treatments of rooting data are given in Table I. It is apparent that cuttings from the lower part of the tree rooted substantially better than those taken from the upper region. Lower cuttings produced more than double the total length of roots of the upper cuttings and the individual roots are appreciably longer. However, position had no effect on the number of roots produced. While there is an apparent difference of 12% in rooting in favour of the treatment with 1000 p.p.m. indolylacetic acid, this is not statistically significant. Treatment with hor-

TABLE I

ROOTING OF NORWAY SPRUCE CUTTINGS 10 WEEKS AFTER PLANTING

	Source of cuttings		Treatment		Necessary difference, 5% level
	Upper	Lower	Talc only	1000 p.p.m. indolylacetic	
Cuttings, rooted, transformed data*	2.7	3.4	2.9	3.2	0.58
Cuttings rooted, %	43	75	53	65	
Number of roots per rooted cutting	1.9	2.2	1.7	2.4	0.67
Total root length per rooted cutting (mm.)	15.2	31.9	22.9	24.2	11.7
Mean root length (mm.)	8.6	14.6	13.1	10.1	5.0

* Data transformed to $\sqrt{x + \frac{1}{2}}$ basis (1).

mone, however, increases significantly the number of roots per rooted cutting; it has no significant effect on the total root length, but reduces significantly the length of individual roots. The last is an observation frequently encountered when root lengths are considered. None of the interactions between treatment and position attained significance.

After a further period of 9 weeks, *i.e.*, 19 weeks in all from the date of planting, the cuttings not rooted at 10 weeks were again examined. The 19-week totals indicated that upper cuttings rooted 48%, lower, 86%; cuttings treated with talc only, 60%, with talc containing 1000 p.p.m. indolylacetic acid, 73%. Analysis of variance showed the effect of position on the tree significant to the 1% level; hormone effects failed to reach significance.

While these results deal with dormant cuttings from only one tree, they do indicate physiological differences in branches from the upper and lower parts of this individual. Further experiments on a number of trees, and extension to other species of spruce, are required to establish the generality of this condition. It will be necessary also to consider the growth of rooted cuttings from these different regions, for it is essential that normal trees be produced; increased ease of rooting from cuttings taken from lower branches will be of no practical value unless such cuttings grow normally and produce properly formed trees.

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FLAX STUDIES

IV. THE PHYSICAL AND CHEMICAL CHARACTERISTICS OF FLAXSEED AT PROGRESSIVE STAGES OF MATURITY[1]

By F. H. Lehberg[2], W. G. McGregor,[3] and W. F. Geddes[4]

Abstract

Flaxseed from plots seeded at Ottawa and Saskatoon over a period of several weeks and harvested at maturity showed no difference in oil content with dates of seeding, but the iodine values tended to increase with late seeding. Experiments conducted with Bison and Redwing grown in 1936 at Brandon, Saskatoon, Edmonton, and Fallis, and with Redwing in 1937 at Brandon, Edmonton, and Fallis, in which the seed was harvested at successive stages of maturity, showed that moisture decreased and dry kernel weight and oil content increased with progressive maturity up to approximately thirty days after flowering. Rate of oil deposition was in some cases extremely rapid, from 80% to 90% of the maximum oil found being deposited by the fifteenth to eighteenth day. Oil content and dry kernel weight reach a maximum several days before visual maturity. Unsaturation proceeds somewhat more slowly and reaches a higher value under climatic conditions favouring slow maturity.

Introduction

In Western Canada, the northern limit of flaxseed production is conditioned by the time required to reach maturity, and it is frequently necessary to harvest the crop before it is fully ripened in order to avoid possible damage from frost. As immaturity is a degrading factor, it is of importance to secure definite information as to its effect on oil content and the drying value of the oil under Canadian cultural conditions.

In other countries some attention has been given to this subject, but only a few investigators have studied progressively the changes in chemical composition as the seed matures; there is little information on the development of unsaturation. Ivanow (9) showed that the oil content of flaxseed harvested at four stages of growth increased with maturity, the values in one year ranging from approximately 4.5% one week after flowering to 35.0% seven weeks later, at which time the seeds were fully ripe. Eyre and Fisher (7) of England reported a range from 21 to 40.9% between the oil content of quite green and ripe seeds separated from plants having green and ripe bolls. On the other hand, at the North Dakota Experimental Station, Washburn (12) found an extreme range of only 38.8 to 40.8% oil for green and mature seeds. Coleman and Fellows (4) reported that immature seeds separated from a number of bulk samples contained an average of 32.6%

oil as compared with 41.4% for the mature seeds. At the South Dakota Experiment Station, Bushey, Puhr, and Hume (3) determined the oil content of flaxseed harvested at five different dates of maturity in 1926 and found it to increase from 29.5%, when approximately one-third of the seeds were greenish in colour, to 36.8% at full maturity, about four weeks later. Dillman (5) carried out a detailed study of the daily seed growth and time of oil formation in the developing seed from flowering to maturity in Minnesota and North Dakota during the years 1926 and 1927; his data show that the most rapid formation of oil occurs between the seventh and eighteenth days after flowering and is correlated with the increase in dry weight. The maximum dry weight and oil content are reached from six to nine days before the seeds are fully ripe, after which there is little change to full maturity. Johnson (10) studied the rate of development of the oil on soils of different productivity in Minnesota. The average oil content of Bison flax showed a fairly uniform increase up to 25 days after flowering, followed by a slight decrease to maturity. The iodine number increased rapidly to 17 days after flowering, remained constant for a short period, then decreased slightly to complete maturity. In the studies reported by Johnson (10), and also by Dillman (5), a large number of flowers were marked and the bolls harvested at a definite period in their development to obtain seed of uniform and definite age. Delayed planting produced a slight reduction in oil percentage during one season but no consistent difference during the other season studied. The iodine number of the oil indicated a slight decrease with delayed planting for Bison but no change for Redwing. In both varieties studied the iodine number at the last date of planting was high.

Under the conditions of the north of Ireland, Eyre (6) and Barker (2) made a study of the development of oil in the seeds of the flax fibre crop with the variety commercially known as "Dutch Blue". They found that the synthesis of the oil takes place slowly up to the eleventh day, after which a period of rapid accumulation sets in, amounting to about 3% per day for some 10 days, when, about 21 days after flowering time, the maximum oil content is reached. The iodine value increased gradually throughout the whole period, the greatest rate of increase taking place after the oil content had reached its maximum value. Robinson (11), working in Michigan with a fibre variety "Saginaw", also found that the oil content of the seed increased with maturity, reaching a maximum before fully ripe.

The study here reported was initiated by the Sub-Committee on Oil Seeds, Associate Committee on Grain Research, and Dominion Department of Agriculture, with the object of investigating the progressive changes in the chemical composition of maturing flaxseed with particular reference to oil content and degree of unsaturation, as measured by iodine value. The co-operating organizations included the Cereal Division, Dominion Department of Agriculture, Ottawa; Grain Research Laboratory, Board of Grain Commissioners, Winnipeg; Department of Agronomy, University of Saskatchewan, Saskatoon; and the Department of Field Crops, University of Alberta, Edmonton.

Experimental

The investigations were begun in 1934 and extended over four crop years. In 1934 and 1935 a "dates of seeding" experiment was conducted in which the bolls were harvested when mature, while in the following two years a study was made of the changes in chemical composition of the seed at progressive stages of maturity.

In all, three varieties, Bison, Redwing, and Novelty, which are the most extensively grown in Canada for seed production, were represented. Bison is characterized by a tall vigorous growth habit, is somewhat late in maturity, and yields medium large seeds. Redwing produces a shorter, stronger stem, matures approximately 10 days earlier, and yields smaller seeds than Bison. Bison has a higher oil content with a lower iodine value than Redwing grown under similar conditions. Novelty is intermediate between Bison and Redwing as regards maturity and size of seed.

For the "dates of seeding" experiment in 1934, Bison, Redwing, and Novelty were seeded at Ottawa, Ontario, in quadruplicate plots, arranged systematically, and trimmed to a rod-row length at harvest time. Seeding commenced as early as land could be prepared and was continued at five-day intervals until ten seedings were made. In 1935, a similar experiment was carried out at Saskatoon in which the variety Bison was sown in duplicate plots at five different dates.

For the study of the physical and chemical characteristics of flaxseed at different stages of maturity in 1936, Bison and Redwing were grown as a quadruplicated, randomized, two-variety, rod-row test at Brandon (Manitoba), Saskatoon (Saskatchewan), and Edmonton and Fallis (Alberta). Sufficient rod-rows were seeded in each plot to provide one for sampling at each stage of maturity.

Harvesting of the bolls commenced at the 15th day after approximately 75% of the plants were in blossom and was continued at three-day intervals until the 36th day after flowering, at all the stations except Fallis, where for practical reasons collections were made at approximately six-day intervals. At each sampling all the bolls from one rod-row were collected, so that there was some variation in the stage of development, because the flax plant continues to flower day by day over a period of 7 to 10 days. In the 1937 experiment, Redwing only was seeded at the same stations, and harvesting was begun at 6 rather than 15 days after 75% of the plants were in blossom. Because of drought, no samples were available from Saskatoon in 1937. In both years, quadruplicate samples of each collection were taken and bulked for laboratory testing.

The bolls harvested at the different stations were forwarded in air-tight containers to Winnipeg for analysis. The bulk of each sample was dried *in vacuo* for 14 hr. at 100° C. without weighing, a small sub-sample being retained for a moisture determination in order that the changes in moisture content with maturity might be followed. Moisture was determined, on

approximately 1 gm. of the whole kernels obtained from the original sub-sample set aside for the purpose, by drying in a vacuum oven at 100° C. for 24 hr.   As some of the high-moisture samples in 1936 had to be discarded on account of spoilage during shipment, and also in order to reduce the possibility of respiration causing changes in chemical composition, the main portion of each sample from the more distant stations (Edmonton and Fallis) in 1937 was dried for a few hours at 100° C. under partial vacuum prior to shipment; but a portion of the freshly harvested material was forwarded in a sealed container for moisture determinations.

The partially dried bulk samples were hand-threshed, and the seed was ground to a fine pulp with a mortar and pestle; the moisture content was determined by the vacuum-oven method, and the dried residue employed for other analytical determinations.   Oil content was determined on from 2 to 10 gm. (depending on the stage of maturity) of the previously dried and ground material, by extracting with anhydrous alcohol-free and peroxide-free ethyl ether in a Soxhlet extractor, with use of Whatman double thickness extraction thimbles.   After 4 hr. of extraction, the samples were re-ground in a mortar with reagent sea-sand and re-extracted for a further 16 hr. at a siphoning rate of one per minute.   To remove traces of starch, the ether extract was filtered through a sintered glass filter and transferred directly to a tared 125 cc. Erlenmeyer flask by suction, and the extraction flask was washed three times with fresh solvent.   The excess ether was distilled off on a water bath maintained at approximately 70° C., the extract dried *in vacuo* for 3 hr. at 98° to 100° C. at a pressure not exceeding 25 mm. mercury, and weighed.   The oil content was expressed on a dry matter basis.   This extraction procedure for determining the oil content was employed in this study rather than the rapid refractometric method described by Geddes and Lehberg (8), as variations in the refractive indices of linseed oil extracted from flaxseed of varying maturity would lead to relatively large errors by the latter method.

In 1934 and 1935, iodine values were determined on cold-pressed oil obtained by means of the Carver Laboratory hydraulic press; but owing to the limited material available in the maturity studies carried out in 1936 and 1937, it was necessary to utilize the ethyl-ether extracts.   Wijs' method, as detailed by the American Association of Official Agricultural Chemists (1), was used in all cases.   Nitrogen was determined on a one-gram sample by the Kjeldahl-Gunning-Arnold procedure essentially as described by the A.O.A.C.   All analyses were conducted in duplicate.

Relation Between Date of Planting and Flaxseed Quality

1934 AND 1935 EXPERIMENTS

The results of the dates of seeding experiments, summarized in Table I, reveal no definite trend in oil content, but there is a definite tendency for the iodine values to increase with delayed seeding, probably because of the cooler weather prevalent during the maturation of the late-seeded plots.

TABLE I

OIL CONTENT AND IODINE VALUE OF MATURE FLAX FROM PLOTS SEEDED AT DIFFERENT
DATES

Variety	Date seeded	Days to maturity	Height, in.	Yield per acre, bu.	Grade	Oil content (dry basis), %	Iodine value of cold pressed oil (Wijs')
Ottawa—1934							
Bison	4 May	86.0	22.0	24.9	1 C.W.	42.3	180.8
	9	84.0	23.0	25.7	1 C.W.	42.6	180.8
	14	83.5	23.0	24.1	1 C.W.	41.7	180.8
	19	83.0	23.7	26.1	1 C.W.	41.1	181.1
	24	82.0	24.2	24.4	1 C.W.	41.1	182.6
	29	84.7	22.7	17.3	1 C.W.	41.2	183.3
	2 June	89.0	21.5	10.5	1 C.W.	41.1	—
	8	89.0	21.0	10.9	1 C.W.	41.1	183.8
	13	97.0	21.2	11.9	1 C.W.	41.5	184.0
	18	92.0	22.7	8.0	1 C.W.	40.7	184.0
Redwing	4 May	82.5	20.5	20.2	1 C.W.	40.3	—
	9	80.5	20.3	20.1	1 C.W.	41.4	189.8
	14	78.2	21.7	18.9	1 C.W.	40.7	190.1
	19	75.7	22.0	21.4	1 C..W	40.6	190.8
	24	74.7	22.7	20.1	1 C.W.	40.7	190.8
	29	76.2	22.5	16.7	1 C.W.	40.7	191.6
	2 June	78.0	19.7	15.8	1 C.W.	40.3	193.5
	8	76.0	19.5	14.1	1 C.W.	40.3	193.5
	13	79.0	17.5	8.0	1 C.W.	40.2	193.9
	18	79.0	19.5	—	1 C.W.	39.9	—
Novelty	4 May	85.0	20.7	22.7	1 C.W.	42.9	182.6
	9	82.5	20.7	23.1	1 C.W.	42.1	183.1
	14	83.2	21.2	—	1 C.W.	41.0	183.1
	19	81.2	21.2	25.4	1 C.W.	41.0	184.6
	24	82.0	21.2	22.0	1 C.W.	41.1	184.7
	29	85.2	20.7	17.4	1 C.W.	41.2	184.9
	2 June	89.0	17.0	—	1 C.W.	40.7	184.9
	8	89.0	17.0	—	1 C.W.	40.9	185.6
	13	97.0	19.0	9.2	1 C.W.	41.8	188.0
	18	92.0	19.7	8.2	1 C.W.	42.5	186.5
Saskatoon—1935							
Bison	24 April	125.0		12.8	1 C.W.	42.6	180.6
	3 May	116.0		15.8	1 C.W.	42.2	183.8
	15	110.0		21.2	1 C.W.	42.9	187.8
	1 June	96.0		8.0	1 C.W.	43.0	190.8
	19	Not mature		8.9	1 C.W.	42.0	192.0

In this connection it is interesting to note that the late-harvested samples of Bison from Saskatoon were higher in iodine number than those from Ottawa, where the average temperature during the late summer is higher.

All varieties made a more vigorous growth when seeded about two weeks after the season opened. Delayed seeding appears to increase the number of days to maturity for Bison and Novelty, the inverse being true for Redwing.

TABLE II

PHYSICAL AND CHEMICAL CHARACTERISTICS OF FLAXSEED AT PROGRESSIVE STAGES OF MATURITY, 1936 EXPERIMENTS

Days after flowering	Date harvested Bison	Date harvested Redwing	Number of days from seeding to harvesting Bison	Number of days Redwing	Appearance Bison	Appearance Redwing	Moisture, % Bison	Moisture, % Redwing	Weight per 100 kernels (dry basis), gm. Bison	Weight Redwing	Oil content (dry basis), % Bison	Oil Redwing	Iodine value (Wijs) Bison	Iodine Redwing
Brandon (seeded May 18)														
15	July 21	July 15	64	58	Green	Green	63.0	65.0	0.33	0.17	32.9	33.0	136.8	139.9
18	24	18	67	61	Green	Green	67.2	69.6	0.38	0.25	34.7	35.1	165.3	161.7
21	27	21	70	64	Brownish	Green	65.2	64.7	0.46	0.32	37.6	37.1	163.3	159.5
24	30	24	73	67	Brown and immature	Brown and immature	45.2	53.4	0.48	0.33	36.5	37.0	169.1	173.1
27	Aug. 2	27	76	70	Immature	Brown and immature	21.0	22.3	0.50	0.35	39.8	37.7	168.4	174.0
30	5	30	79	73	Immature	Immature	10.6	12.2	0.53	0.37	38.2	37.0	172.8	174.6
33	8	Aug. 2	82	76	Nearly mature	Mature	7.7	8.1	0.54	0.39	38.0	37.3	175.3	178.7
36	11	5	85	79	Nearly mature	Mature	7.6	7.8	0.54	0.39	38.6	37.8	173.8	179.3
Saskatoon (seeded May 13)														
15	July 18-19	July 18	66.5	66	Green	Green	67.3	65.3	0.22	0.22	35.0	35.4	130.2	142.4
18	21-22	21	69.5	69	Green	Green and brown	55.2	52.7	0.27	0.29	36.3	36.2	159.6	165.0
21	24-25	24	72.5	72	Green and immature	Brown and immature	28.5	26.8	0.33	0.36	36.8	36.2	182.4	182.4
24	27-28	27	75.5	75	Immature	Brown and immature	10.6	10.9	0.38	0.39	38.2	38.0	180.7	181.9
27	30-31	30	78.5	78	Immature	Brown and immature	10.2	9.8	0.39	0.40	38.6	39.0	182.2	187.4
30	Aug. 2-3	Aug. 2	81.5	81	Mature	Fully mature	7.6	7.8	0.39	0.40	39.8	39.0	181.8	182.7
33	5-6	5	84.5	84	Mature	Fully mature	6.6	—	0.39	0.40	39.4	38.2	185.5	185.6
36	8-9	8	87.5	87	Mature	Fully mature	6.8	—	0.40	0.40	39.4	37.8	186.1	186.8

TABLE II—Concluded

PHYSICAL AND CHEMICAL CHARACTERISTICS OF FLAXSEED AT PROGRESSIVE STAGES OF MATURITY, 1936 EXPERIMENTS—Concluded

Days after flowering	Date harvested		Number of days from seeding to harvesting		Appearance		Moisture, %		Weight per 100 kernels (dry basis), gm.		Oil content (dry basis), %		Iodine value (Wijs')	
	Bison	Redwing	Bison	Redwing	Bison	Redwing	Bison	Redwing	Bison	Redwing	Bison	Redwing	Bison	Redwing
Edmonton (seeded May 12)														
15	July 16-17	July 13-14	65-66	62-63	Green	Green	79.0	76.4	0.16	0.13	11.1	11.4	125.4	122.9
18	19-20	16-17	68-69	65-66	Green	Green	78.1	76.5	0.22	0.17	18.4	20.5	136.0	139.7
21	22-23	19-20	71-72	68-69	Green	Green	72.8	73.5	0.25	0.23	33.1	33.8	144.4	145.5
24	26	22-23	75	71-72	Green	Green	73.0	71.2	0.33	0.29	36.4	36.9	153.8	159.3
27	28-29	26	77-78	75	Green	Green and brown	67.0	71.7	0.41	0.37	40.4	38.8	162.6	169.6
30	31, Aug. 1	28-29	80-81	77-78	Green and brown	Brown and immature	—*	62.2	0.53	0.41	41.1	39.4	180.4	182.4
33	Aug. 3-4	31, Aug. 1	83-84	81	Brown and immature	Brown and immature	52.0	48.3	0.53	0.42	41.8	40.3	182.7	185.5
36	7	Aug. 3-4	87	83-84	Brown and immature	Brown and immature	56.6	42.7	0.52	0.43	41.9	40.3	184.3	187.8
Fallis (seeded May 19)														
15	July 26-27-28	July 21-22	68-69-70	63-64	Green	Green	77.7	79.3	0.15	0.14	20.1	21.8	—*	—*
21	Aug. 1-2	27-28	74-75	69-70	Green	Green	75.2	74.8	0.31	0.26	29.9	31.6	—*	—*
27	7-8	Aug. 3	80-81	76	Green	Green	74.5	—*	0.38	0.29	32.7	35.2	—*	—*
33	14	8-9-10	84-87	81-82-83	Green	Green and brown	73.2	40.5	0.53	0.33	41.4	39.4	—*	—*
39	19-20	15-16	92-93	88-89	Green and brown	Green and brown	70.0	52.6	0.58	0.41	41.5	38.7	—*	—*
45	26-27	20-21	99-100	93-94	Green and brown	Immature	63.0	—*	0.58	0.44	41.3	40.2	—*	—*
51	31, Sept. 1-2	26-27-28	104-105-106	99-100-101	Green and brown	Immature	—*	49.2	—	0.45	42.0	—	—*	—*
57	Sept. 7-8	Sept. 2	111-112	106	Very immature	Immature	—*	—*	—	—	42.0	—	—*	—*

* Not tested because of slight spoilage.

Varieties which do not reach a certain stage of maturity before the end of the season are retarded by second growth. As previous work had shown, later seeding usually produces an appreciable decrease in yield.

Physical and Chemical Characteristics of Flaxseed at Progressive Stages of Maturity

1936 EXPERIMENTS

The results of the 1936 experiments are recorded in Table II, and represented graphically for each station excepting Fallis in Figs. 1, 2, and 3. It will be observed that, as indicated by oil content, the plants grown at Brandon and Saskatoon were considerably matured prior to the 15th day after flowering, when the first samples were collected. At all stations, both
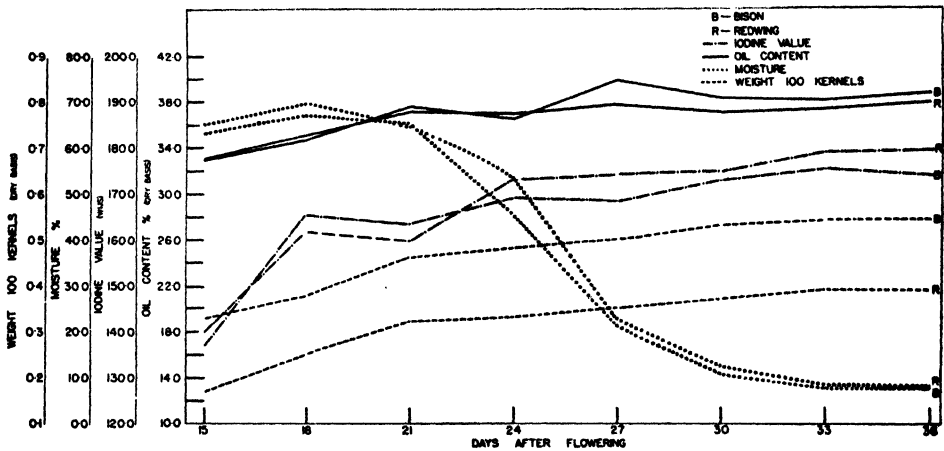

FIG. 1.   Various characteristics of Bison and Redwing flax grown at Brandon, Manitoba, in 1936 and harvested at different stages of maturity.
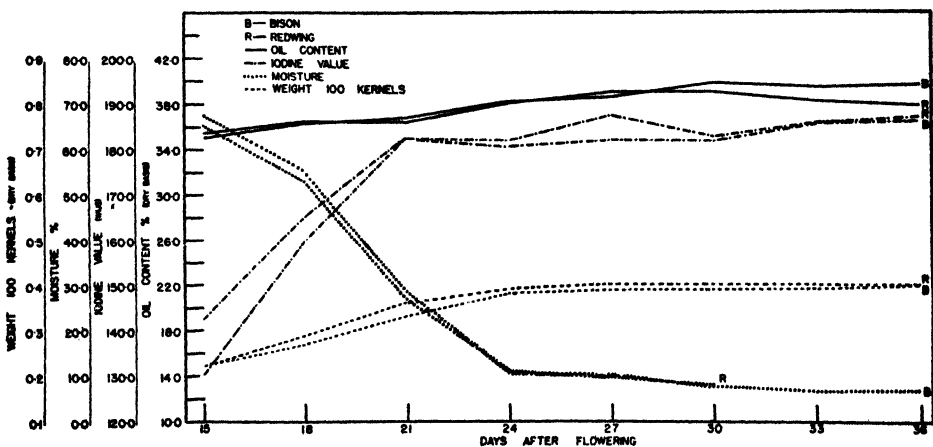

FIG. 2.   Various characteristics of Bison and Redwing flax grown at Saskatoon, Saskatchewan, in 1936 and harvested at different stages of maturity.

varieties showed a decrease in moisture, increase in dry kernel weight and increase in oil content with progressive maturity up to approximately 30 days after flowering; the increase in iodine value continues after maximum dry kernel weight and oil content is attained.
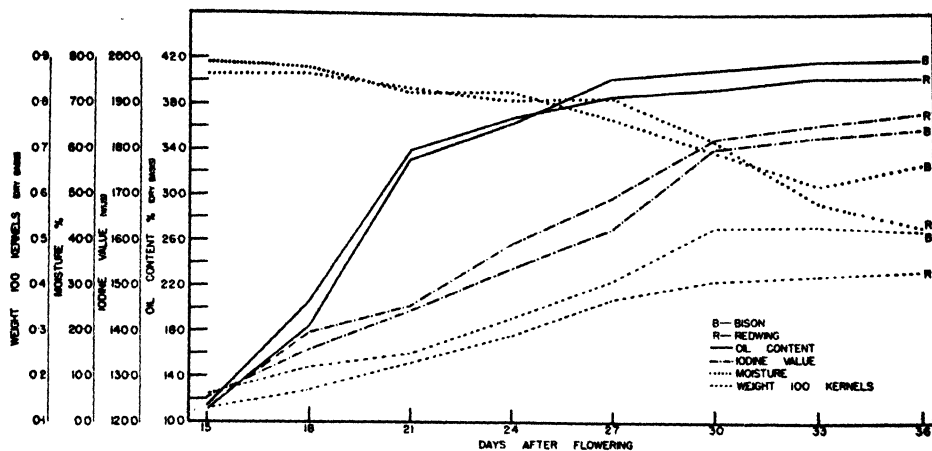

FIG. 3. *Various characteristics of Bison and Redwing flax grown at Edmonton, Alberta, in 1936 and harvested at different stages of maturity.*

These changes with maturity are more gradual for the Edmonton and Fallis series, and are probably associated with cooler weather and higher soil moisture at these points. The oil contents of the Edmonton samples were approximately 11% at the 15th day after flowering, increasing rapidly to 33% by the 21st day; as the oil content at the last harvesting was approximately 42%, 52% of the final oil was deposited within six days. After the 21st day the oil content increased more gradually and at the 27th day had reached 96% of the final value; at this time the kernels were still immature in appearance and had the characteristic greenish brown colour. The increase in iodine value at Edmonton is more gradual than the increase in oil content and continues throughout the entire collection period. At the 15th day the iodine values were 125.4 and 122.9 for Bison and Redwing respectively, increasing to 184.3 and 187.8 by the 36th day. At the final sampling the oil content of Bison was 1.6% higher than Redwing while the iodine value was 3.5 units lower.

At Brandon and Saskatoon, drier weather conditions prevailed and the days to maturity were less than at Edmonton and Fallis. At the initial sampling approximately 85 to 90% of the oil finally found was already present, and the degree of unsaturation was also well advanced. For Brandon, the iodine values for Bison and Redwing increased from 136.8 and 139.9 units respectively at 15 days after flowering, to 173.8 and 179.3 units at 36 days, at which time the samples appeared mature.

At all stations, Bison yielded a higher final oil content and lower iodine value than Redwing; these are recognized varietal differences.

1937 EXPERIMENTS

The analytical data for the 1937 experiments are summarized in Table III and graphically represented in Figs. 4, 5, and 6.

The general trends were similar to those observed in 1936; as maturity progressed moisture content rapidly decreased and the dry kernel weight, oil content, and iodine value increased. Oil deposition commenced very early in kernel development, approximately 17, 32, and 23% being present by the sixth day after flowering in the Brandon, Edmonton, and Fallis series respectively. It is extremely doubtful whether the estimation of the flowering stage was similar at the three stations, since from general climatic conditions one would expect the Edmonton and Fallis series to be lower in oil content than the Brandon series at this early state of maturity. This is again suggested by the comparative iodine values of 98, 129, and 120 units for the initial sampling at the above respective stations. As in 1936, the iodine values at all stations increased somewhat more slowly than oil deposition, reaching a maximum at a later stage of maturity. Visually, the Brandon, Edmonton, and Fallis samples were approaching maturity at 33, 40, and 54 days, respectively, after flowering. Complete maturity, as evidenced by the characteristic reddish-brown colour of Redwing, occurred on the 52nd and 58th days after flowering at Brandon and Edmonton, while the Fallis samples never reached full visual maturity; yet the iodine values for Fallis were the highest.

The fluctuations in the oil content of successive collections appear to follow the precipitation that occurred during the intervals between samplings, the oil content tending to increase after rain and to drop following a dry period. Iodine value does not appear to be similarly affected.

The nitrogen content of the seeds showed no pronounced trends with maturity in the Edmonton and Fallis series, but for Brandon there was a fairly consistent increase to the 24th day after flowering.

Discussion

The slight decrease in oil content and iodine number with delayed planting suggested by Johnson (10) was not apparent in these data. Oil content depends to some extent on maturity, and if all samples are mature not much variation in oil content is to be expected. However iodine number in each instance increased with delayed planting.

The results in regard to the rapidity with which oil is deposited in the developing kernel correspond quite well with those of other workers reported in the literature. Even at the sixth day after flowering there is an appreciable percentage of oil present, and in most cases approximately 80 to 85% of the maximum oil content found is present by the 15th to 18th day. The oil content increases with increasing dry kernel weight, both reaching a maximum several days before the sample is visually mature; this time varies with the environmental conditions. The data therefore indicate that harvesting the flax before it is completely ripe will not result in a decrease in oil yield.

TABLE III

PHYSICAL AND CHEMICAL CHARACTERISTICS OF REDWING FLAXSEED HARVESTED AT DIFFERENT STAGES OF MATURITY, 1937 EXPERIMENTS

Days after flowering	Date harvested	Number of days from seeding to harvesting	Appearance	Precipitation, in.			Moisture, %	Weight per 100 kernels, gm.	Oil content (dry basis), %	Iodine value (Wijs)	Nitrogen (dry basis), %
				1 day	2 days	3 days					
Brandon (seeded May 25)											
6	July 15	51	Green and very thin	—	—	.01	80.5	.12	17.0	98.3	2.93
9	18	54	Green and very thin	—	—	—	79.0	.15	23.9	120.4	3.23
12	21	57	Green and thin	—	—	.02	76.8	.20	30.4	126.3	3.83
15	24	60	Green and thin	.33	—	.09	72.5	.31	33.0	136.2	3.73
18	27	63	Green and plump	—	.08	—	64.8	.38	35.1	142.0	4.16
21	30	66	Green	—	.26	.02	64.5	.41	36.8	148.8	4.27
24	Aug. 2	69	Green	1.15	1.13	—	59.7	.46	41.3	152.0	4.47
27	5	72	Green	—	—	—	57.5	.45	38.4	176.0	4.40
30	8	75	Green and brown	—	—	1.17	60.2	.43	37.2	185.5	4.40
33	11	78	Immature and brown	.82	—	.11	55.3	.45	41.4	187.7	4.45
36	14	81	Immature and brown	—	Trace	.17	22.4	.49	40.1	189.2	4.50
39	17	84	Immature and brown	—	—	—	29.0	.46	38.8	184.6	4.45
42	20	87	Immature and brown	—	.29	—	44.0	.48	42.0	189.5	4.35
45	23	90	Immature and brown	—	—	—	22.2	.48	41.5	182.7	4.38
48	26	93	Immature and brown	—	—	—	16.4	.46	40.3	183.2	4.26
52	30	97	Brown, practically mature		Aug. 27, trace		17.8	.46	41.2	185.6	4.55
55	Sept. 2	100	Brown and mature	—	—	—	17.9	.46	40.6	186.3	4.26
58	5	103	Brown and mature	—	—	.30	14.5	.45	40.8	191.2	4.48
61	8	106	Brown and mature	—	—	—	10.0	.46	40.4	187.7	—
64	11	109	Brown and mature	—	—	—	8.0	.46	39.1	188.2	4.16
67	14	112	Brown and mature	—	—	—	5.6	.45	—	187.0	4.23
70	17	115	Brown and mature	—	—	—	5.4	.45	38.0	—	4.34
Edmonton											
6	July 24		Green and thin	.08	—	—	76.0	.21	32.0	128.8	3.69
9	27		Green and thin	—	.04	—	77.2	.29	35.2	139.0	3.70
12	30		Green and thin	.10	.30	.80	74.2	.28	37.6	162.3	3.73

TABLE III—*Concluded*

PHYSICAL AND CHEMICAL CHARACTERISTICS OF REDWING FLAXSEED HARVESTED AT DIFFERENT STAGES OF MATURITY, 1937 EXPERIMENTS—*Concluded*

| Days after flowering	Date harvested	Number of days from seeding to harvesting	Appearance	Precipitation, in.			Moisture, %	Weight per 100 kernels, gm.	Oil content (dry basis), %	Iodine value (Wijs')	Nitrogen (dry basis), %
				1 day	2 days	3 days					
Edmonton—Concluded											
15	Aug. 2		Green and thin	.20	—	.01	72.3	.32	39.1	170.0	3.62
18	5		Green and thin	—	.06	.10	73.4	.33	37.5	182.0	4.05
21	8		Green	.04	—	.04	69.0	.38	40.3	187.0	4.11
24	11		Green	—	—	—	68.8	.40	40.8	196.0	4.08
28	15		Green	.12	.02	.24	65.2	.41	42.6	191.0	4.07
31	18		Green and brown	—	.01	.02	66.0	.38	39.7	189.5	3.75
34	21		Green	—	—	—	63.7	.41	43.3	190.0	3.92
37	24		Brown and some green	—	.18	—	61.8	.43	41.8	192.0	3.88
40	27		Immature and brown	—	.12	—	55.3	.48	41.2	185.0	4.06
43	30		Immature and brown	.02	Trace	.24	61.9	.49	43.6	179.8	4.02
46	Sept. 2		Immature and brown	—	.12	—	55.4	.44	41.6	180.2	4.05
49	5		Immature and brown	—	—	—	55.3	.49	42.7	195.5	4.16
52	8		Immature and brown	—	—	—	46.7	.50	42.4	197.5	3.83
55	11		Immature and brown	.04	—	—	42.0	.49	40.8	188.9	4.15
58	14		Practically mature	—	—	—	35.5	.52	41.5	184.5	4.18
65	17		Practically mature	—	—	—	35.0	.46	40.6	186.3	4.05
72	24		Practically mature	Sept. 20 .74	Sept. 21 .28	Sept. 22 .30	27.3	.48	42.2	189.8	3.55
Fallis											
6	July 30		Green	Not recorded			72.8	.26	23.6	120.4	3.61
12	Aug. 5		Green				69.8	.41	35.7	136.7	4.05
18	11		Green				76.0	.42	40.5	169.0	3.87
24	17		Green				70.0	.40	39.8	183.2	3.62
30	23		Green and brown				38.1	.40	39.0	194.6	3.35
36	29		Green and brown				66.4	.44	39.0	189.5	3.55
42	Sept. 3		Green and brown				46.6	.42	38.7	191.3	3.55
48	9		Green and brown				21.8	.45	40.6	199.3	—
54	15		Brown and immature				55.4	.44	40.0	196.5	3.28
61	22		Brown and immature				49.1	.46	39.3	194.0	3.94
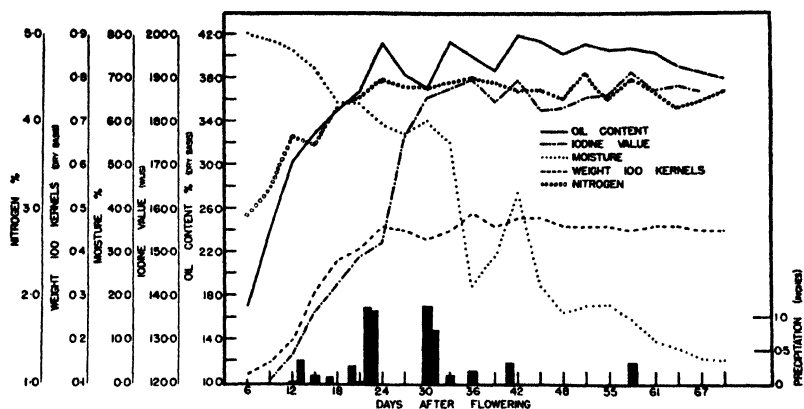
FIG. 4. Various characteristics of Redwing flax grown at Brandon, Manitoba, in 1937 and harvested at different stages of maturity. Precipitation also shown.
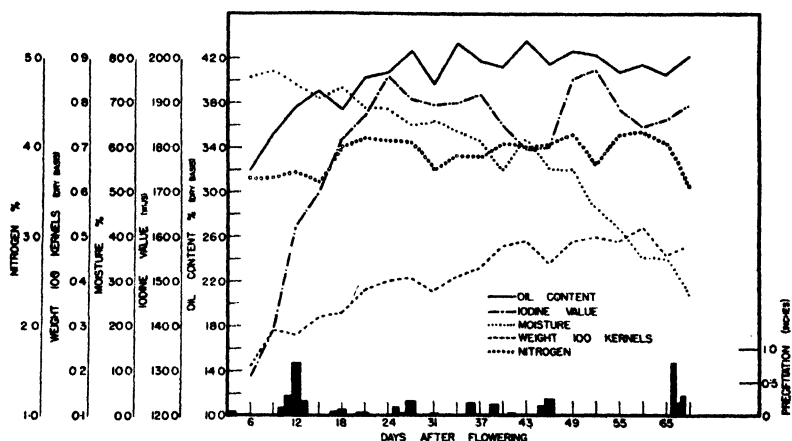

FIG. 5. Various characteristics of Redwing flax grown at Edmonton, Alberta, in 1937 and harvested at different stages of maturity. Precipitation also shown.
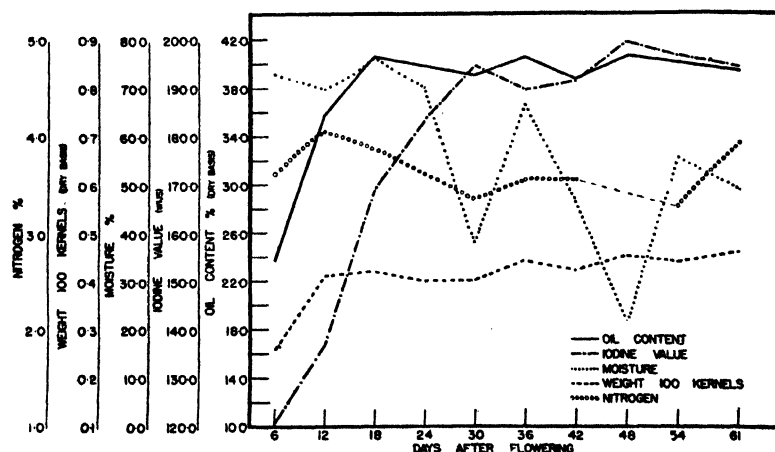

FIG. 6. Various characteristics of Redwing flax grown at Fallis, Alberta, in 1937 and harvested at different stages of maturity.

While there are some inevitable incongruities in the data, it is evident that the development of unsaturation proceeds somewhat more slowly than oil deposition and tends to reach a higher final value under climatic conditions favouring slow maturity. The results, however, are not sufficiently conclusive to warrant the statement that immaturity tends to result in lower iodine values, although the indications are in this direction.

The ability of flax to stand in the field beyond maturity without shattering is an important feature in harvesting operations, especially since the introduction of the combine harvester. Collections from the crop at Saskatoon in 1936 and at Brandon in both 1936 and 1937 would indicate that no deterioration in oil content or iodine number might be expected from this practice.

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SEPTORIA CANKER OF INTRODUCED AND NATIVE HYBRID POPLARS[1]

By J. E. Bier[2]

Abstract

Studies have shown that *Septoria musiva* Peck, a North American fungus which occurs commonly as a leaf-spotting parasite on native poplars, produces cankers, in addition to leaf injury, on certain introduced poplars (*Populus Rasumowskyana* Schneid., *P. Petrowskyana* Schneid., and *P. berolinensis* Dipp.), and the native hybrids, Northwest and Saskatchewan poplar.

Field observations and inoculation experiments demonstrated that most of the inoculum for spring infection arises from ascospores of a *Mycosphaerella* stage, and that the fungus enters the stems through mechanical wounds, uninjured lenticels, leaf petioles, or stipules. Incipient cankers occur in the bark of the current year's wood, soon girdling leading and side shoots. They later spread from lateral branches into the main stem, developing into perennial cankers which ultimately girdle and kill the trees.

Introduction

In recent years the attention of the author has been directed to a destructive canker of certain strains of poplar growing in the vicinity of Indian Head, Saskatchewan, and at the Petawawa Forest Experiment Station, Ontario. The disease was reported on *Populus Rasumowskyana* Schneid., *P. Petrowskyana* Schneid., *P. berolinensis* Dipp., Northwest poplar, and Saskatchewan poplar. The first three are introduced species of hybrid origin, which collectively have been called Russian poplar, while Northwest and Saskatchewan poplars are considered native hybrids between balsam poplar (*P. tacamahaca* Mill.) and cottonwood (*P. balsamifera* L.). Because of their hardiness, rapid growth, and ease of propagation, these species have been used extensively for windbreak and shelterbelt plantings in the Prairie Provinces.

When first observed it was noted that the cankers did not resemble those associated with *Hypoxylon, Cytospora*, or *Dothichiza*, the commonly recognized canker-producing fungi on poplar. Isolations from the bark of cankers of all ages repeatedly produced fruiting cultures of a species of *Septoria*. At this point in the investigation it was found that Mr. J. L. Van Camp had previously carried out experiments on the canker of Russian poplar, and had recorded a *Septoria* as the causal agent. A brief summary of Van Camp's work was published in the Report of the Director of Forestry for the year 1929–30 (1). These investigations were not continued after 1930, and no further research was carried out until 1936, when the problem was undertaken by this Service.

During the summer of 1938 the disease was investigated at Indian Head, Sask., and there is no doubt that the cankers and fungus studied in Ontario are the same as those previously described by Van Camp in Saskatchewan.

The Disease

Historical Sketch of the Hosts and Disease

Russian poplars have been grown in Canada for the last 40 to 50 years. Saunders (7) in 1904, describing trees tested in Manitoba and the Northwest Territories, mentioned Russian poplar as a very hardy and fast-growing tree of value to the Northwest country. Johnson (2) in 1923, writing on tree planting in the Great Plains region, made the following statement with respect to Russian poplar: "It is hardy to winter cold and resists the ordinary amount of drought, but is subject to attack by canker, a disease which soon girdles the tree, generally near the base or at a crotch, and kills the portion above the canker. The disease is quite common on all Russian poplars grown in this region. For that reason they are not recommended for general planting."

In 1928, Munro (3) reported the presence of Russian poplar canker in Saskatchewan, and observed that the disease was more severe on higher planting sites. In 1930, Van Camp (1) recorded investigations on the disease which were carried out at Indian Head, Sask. A *Septoria*, identical with the fungus occurring as a leaf parasite on Russian poplar, was reported to be a wound parasite on the stems of *P. Petrowskyana* and Northwest poplar. Cankers were produced experimentally from wound inoculations made under greenhouse conditions, and methods were recorded for the control of the disease by the utilization of clean cuttings and clean planting sites. In 1933, Ross (6) advised against planting Russian poplars in large numbers on account of their susceptibility to canker and insect borers.

EXTENT OF DAMAGE
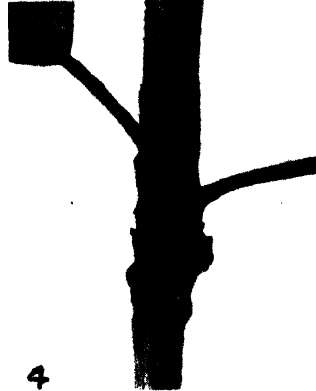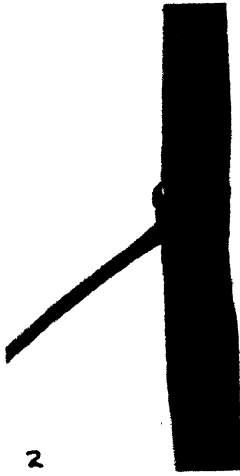
Canker Stage

In Ontario, field observations on Septoria canker have been confined to a 13-year-old plantation of *P. Rasumowskyana* at the Petawawa Forest Experiment Station. On this area all trees are infected, each having a number of lesions on the trunk and branches which ultimately girdle and kill the affected parts. The average condition of the trees is illustrated in Plate I, which shows the upper half of a tree 17 ft. high. The host in turn forms new branches from adventitious buds on the stem, and frequently sucker shoots that may develop into trees are produced from the stumps. After 13 years of growth the trees are 10 to 20 ft. high, much branched, with from one to three stems arising from the original root system of each planted cutting.

Shelterbelts and plantations of *P. Petrowskyana*, Northwest, and Saskatchewan poplars in the prairie region of Manitoba, Saskatchewan, and Alberta have also been examined for cankers. The disease incidence coincided with the distribution of susceptible hosts, and on many occasions, large plantings established during the last 20 years had been practically eliminated as the result of cankers. In the vicinity of Edmonton, Alta., Russian poplars were in a much healthier condition than those observed in the southern part of the Prairie Provinces. Cankers were present, but the more vigorous growth of the trees resulted in the rapid production of secondary periderm around the lesions, which apparently was sufficient to inhibit the further advance of the pathogen.

PLATE I

Septoria canker on P. Rasumowskyana at the Petawawa Forest Experiment Station, Ont. The photograph shows the upper half of a 12-year-old tree which was approximately 17 ft. high. Note the two cankers on the main stem. The uppermost lesion has recently girdled the leader. The stem swelling immediately above the lower canker indicates a lesion on the opposite side of the trunk. Also observe the newly killed branch (the second from the ground on the right), and the responsible canker adjacent to the trunk. The leaves often persist on the dead parts until the following spring.

PLATE II

Leaf Spot Stage

Leaf lesions were universally present on trees susceptible to the canker stage (Plate IV). The injury was more severe on the leaves of lower branches, a condition possibly correlated with the discharge of ascospores from the perfect fruiting bodies produced on the overwintered leaves. Although no defoliation was noted as a direct result of leaf injury, the large amount of leaf area killed would appreciably reduce the photosynthetic activity, and in this way have a decidedly unfavourable effect upon the annual increment.

DISEASE DEVELOPMENT

Most of the primary inoculum for the spring infection of leaves and stems arises from the abundant perithecia on the overwintered leaves. In addition to those on the ground, the perfect stage was found on leaves persisting on branches that had been killed during the previous growing season. Both perithecia and pycnidia containing viable spores were collected on lesions that had girdled the twigs developed during the previous year. Therefore, the spring dissemination of the pathogen is not wholly dependent on the ascospores. Ascospore discharge occurs from the time the buds open until the development of the new growth is complete.

On the Branches and Stems

Field observations and artificial inoculations have shown that cankers originate in the bark of twigs of the current year. The fungus may enter the host through mechanical wounds, uninjured lenticels, stipules, or leaf petioles (Plate III). By the middle of June under field conditions a very conspicuous symptom of the disease is the presence of one or more dead leaves on the leaders (Plate III, Fig. 1), at the ring scars (Plate II, Fig. 3), or on the axillary

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PLATE II

Septoria canker on P. Rasumowskyana at the Petawawa Forest Experiment Station, Ont.

FIG. 1. *Stem canker arising at a leaf axil.* ×¾. *Note the dead leaf hanging down from the centre of the blackened diseased area.*

FIG. 2. *Enlargement of the lesion shown in Fig. 1.* ×2. *Note the pycnidia in the enclosed paler area of the lesion.*

FIG. 3. *Stem canker at the junction of the current and last year's growth.* ×¾. *Observe the small leaf on the left which was killed before reaching maturity.*

FIG. 4. *Enlargement of the lesion shown in Fig. 3.* ×2. *Note the blackened diseased area spreading into 2-year-old bark on the left.*

FIG. 5. *Canker on a 5-year-old stem.* ×¾. *Observe the swelling of the stem at the upper margin of the canker.*

FIGS. 6 TO 9. *Stages in the development of a perennial stem canker, arising from an initial infection in the current growth of an axillary shoot. The four lesions were collected from different positions on the same main stem.* ×¾.

FIG. 6. *The current growth of an axillary shoot has become infected. Note the dead, shrivelled leaves on the black, dwarfed, axillary shoot which was produced during the second growing season of the stem. The infection has not as yet spread into the main stem.*

FIG. 7. *Canker condition on 3-year-old stems. The axillary shoot has fallen off, and the fungus has entered the main stem. Note the black, diseased bark below the branch scar, and the stem swelling around the diseased area.*

FIG. 8. *Canker condition on 4-year-old stems. Observe the pronounced stem swelling at the canker margins, and the spread of the infection into healthy bark at the top, central portion of the lesion.*

FIG. 9. *Canker condition on 5-year-old stems. Note the successive callous layers formed during the third and fourth growing seasons. The diseased bark at the top of the canker has been secondarily attacked by Cytospora. The lesion had just completed girdling the stem.*

branches produced on 2-year-old stems (Plate II, Fig. 6). Closer examination reveals stem lesions at the bases of the dead leaves (Plate II, Figs. 2 and 4). The diseased bark is usually black, frequently enclosing yellowish to white areas in which small pycnidia may be found (Plate II, Fig. 2, and Plate III, Fig. 2). The cankers girdle and kill the leader and axillary branches during the first growing season, but the spread of the disease from axillary branches into the main stem as a rule does not occur until the following year, *i.e.*, the third growing season of the stem (Plate II, Fig. 7). On entering the main stem, the pathogen produces a canker that is perennial in character. Each year considerable malformation of the stem arises at the margin of the lesion (Plate II, Figs. 8 and 9). In general, the cankers succeed in girdling the stems during their fourth or fifth year of growth.

Pycnidia and perithecia are found on infections on the current year's wood (Plate V, Fig. 1), but only on rare occasions have they been observed in cankers occurring on wood two or more years of age.

It is important to note that the bark of dead trees and cankered areas is secondarily attacked by *Cytospora chrysosperma* (Pers.) Fries (Plate II, Fig. 9), a fungus parasitic on some species of poplar. Isolations from the diseased bark at the margin of older lesions not infrequently produce cultures of *Cytospora*, and it is possible that advanced cankers may result from a combined attack of *Septoria* and *Cytospora*.

On the Leaves

The first leaf lesions appear from three to four weeks after the opening of the buds, and are usually confined to leaves on the lower branches. The number of lesions multiplies rapidly during the growing season, and the infection becomes more or less general throughout the trees. The fungus produces

PLATE III

Cankers resulting from artificial inoculations.

FIG. 1.   *Stem canker at a leaf axil of P. Rasumowskyana.* ×¾.   *On April 22, 1938, spores were placed at approximately the mid-point on the leaf petiole.   A petiole lesion developed and the fungus was observed to pass down the petiole into the stem.   The photograph was taken on June 15.*

FIG. 2.   *Stipule infection on Northwest poplar.* ×¾.   *On June 6, 1938, spores were placed on the green stipules at the leaf axil.   Lesions formed on the stipules, and stem cankers developed from their bases.   Note the pycnidia on the canker.   The photograph was taken on June 30.*

FIG. 3.   *Lenticel infection on Northwest poplar.* ×¾.   *Two weeks after spores were painted on the stem, slight swellings were evident around some of the lenticels.   Almost immediately elongate, oval, black areas of diseased bark were produced.*

FIG. 4.   *Multiple infection resulting from an inoculation on an unwounded stem of Northwest poplar.* ×¾.   *On June 6, 1938, spores were painted on the leaf petioles, stipules and stem.   The photograph was taken on June 26, and at this time stipule, lenticel, and petiole infection was apparent.   Note the infected lenticel near the base of the cutting, and the local swelling of the entire stem arising from the infection of a number of lenticels.*

FIG. 5.   *Stem wound inoculation on cottonwood.* ×¾.   *A small lesion was produced as a result of the inoculation.   The diseased area was soon delimited by secondary periderm tissue of the host, and no further canker growth occurred.*

FIG. 6.   *Wound inoculation on P. Rasumowskyana.* ×¾.   *Canker present 15 days after inoculation.*

FIG. 7.   *Wound inoculation on Northwest poplar.* ×¾.   *Canker present 20 days after inoculation.   Note the pycnidia in the paler areas of diseased bark.*

PLATE III

PLATE IV
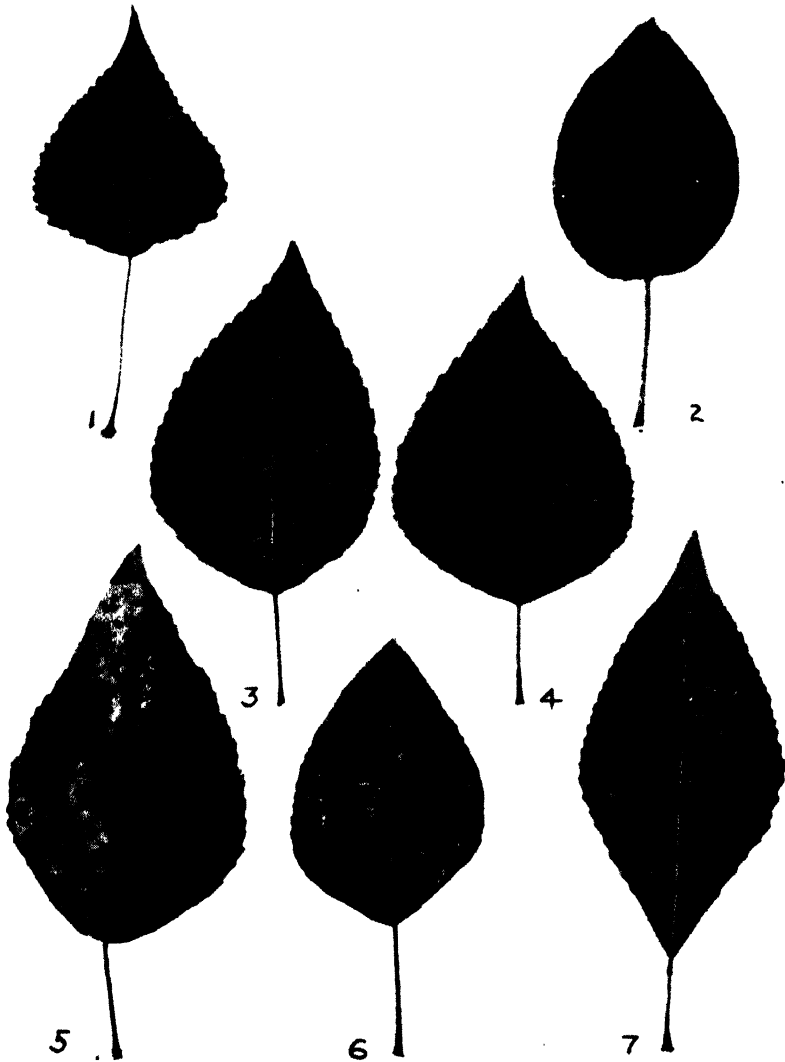
Leaf lesions resulting from artificial inoculations. ×¾. *1. P. balsamifera. 2. P. tacamahaca 3. Northwest poplar. 4. Saskatchewan poplar. 5. P. Rasumowskyana. 6. P. Petrowskyana. 7. P. berolinensis.*

necrotic spots of various sizes which often coalesce to involve large areas of the leaf. The individual lesions may be either circular or angular, and are brown with yellowish to white centres (Plate IV). The angular spots are frequently limited by the veins. Small, black pycnidia are evident, scattered throughout the lesions on both leaf surfaces (Plate V, Fig. 2).

The Causal Fungus

TAXONOMY AND MORPHOLOGY

The Imperfect Stage

The fungus associated with the leaf lesions and stem cankers of Russian, Northwest, and Saskatchewan poplars, agrees with Peck's (4) original description of *Septoria musiva*,* and the type specimen collected on *P. monilifera* (cottonwood), at Albany, N.Y.

The pycnidia are embedded in the tissues, with the ostioles projecting through the leaf epidermis or bark periderm (Plate V, Figs. 1, 2, and 3). In longitudinal section the pycnidial wall is evident, with the conidia borne along the base and sides of the pycnidium (Plate V, Fig. 4). The pycnidia vary in width from 64 to 120 μ, and in height from 68 to 129 μ, with an average respectively of 88 by 96 μ.

When leaves or stems bearing pycnidia are placed in a moist chamber, the spores are discharged from the fruiting bodies in the form of long, curled, pinkish cirri. The conidia are hyaline, continuous to four- (mostly two-) septate, measuring from 17.2 to 57μ long. No marked variation was encountered in the width of the conidia, which varied from 3 to 4 μ (Plate V, Fig. 5). In Table I more detailed conidial measurements are given for the fungus on cottonwood and the other hosts susceptible to canker.

TABLE I

CONIDIAL MEASUREMENTS (IN μ)

Host	From pycnidia on cankers			From pycnidia on leaves		
	Longest	Shortest	Average	Longest	Shortest	Average
Russian poplar	55.6	21.5	37.9 (100)	51.6	17.2	31.6 (160)
Northwest poplar	54.7	25.8	41.7 (40)	47.3	25.8	34.9 (40)
Saskatchewan poplar	51.6	30.1	38.7 (40)	43.0	25.8	36.9 (40)
Cottonwood	–	–	–	49.5	21.5	36.1 (90)

The figures in brackets after each average designate the total number of measurements taken.

In late August, September, and October, smaller embedded pycnidia are formed on the lesions. Apparently these are spermogonial structures. The fruiting bodies are filled with rod-shaped, one-celled, hyaline spores, measuring from 4 to 7 by 1 to 2 μ.

* *Dr. G. E. Thompson confirmed the author's identification of S. musiva on Russian poplar and cottonwood.*

The Perfect Stage

In September 1937, *Septoria*-infected leaves of Russian poplar and cotton-wood were placed in wire baskets and overwintered out-of-doors. Some leaves were brought into the laboratory early in May 1938, and 24 hr. after they had been placed in a moist chamber, abundant ascospore discharge was obtained from small, globose perithecia embedded in the leaf tissues. The ascospores germinated and formed mycelial colonies that later produced the *Septoria* stage. Subsequent inoculation experiments confirmed the connection between the perfect and imperfect stages of the fungus. The asci, which contain eight spores, are cylindrical, short stipitate, from 51 to 73 μ long by 12 to 17 μ wide. The ascospores are hyaline, one-septate, measuring from 17 to 24 μ long by 4 to 6 μ wide. The description of the perfect stage agrees essentially with a new species of *Mycosphaerella* described by Thompson (8) as the perfect stage of *S. musiva*.

HOSTS AND GEOGRAPHICAL DISTRIBUTION

An important feature of *S. musiva* is that it is considered to be indigenous to North America and is not known to occur elsewhere. The preceding section has demonstrated that the pathogen is a leaf parasite on cottonwood in addition to the canker-susceptible hosts. During this investigation the fungus has also been found producing leaf injury on one or more of the following trees: balsam poplar (*P. tacamahaca* Mill.), trembling aspen (*P. tremuloides* Michx.), Balm of Gilead (*P. candicans* Ait.), and western balsam poplar (*P. trichocarpa* Torr. and Gray) in the provinces of Quebec, Ontario, Manitoba, Saskatchewan, and Alberta.

It is apparent, then, that the fungus producing the cankers on Russian, Northwest, and Saskatchewan poplars occurs as a leaf-spotting parasite on several native poplars distributed throughout the Dominion of Canada.

CULTURAL STUDIES

When suspensions of conidia or ascospores in water are poured on the surface of potato dextrose agar, the spores germinate at once, and the colonies appear within 48 hr. at room temperature. The colonies are white at first,

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PLATE V

FIG. 1. *Septoria stage on a current-year twig infection.* ×8. *Note the conidia discharged in the form of cirri.*

FIG. 2. *Leaf lesion on P. balsamifera.* ×5. *Note the angular form of the necrotic area and the pycnidia scattered throughout. In some instances it is possible to see cirri extending out from the pycnidia.*

FIG. 3. *Longitudinal section through a leaf demonstrating the embedded pycnidium breaking through the leaf epidermis.* ×180.

FIG. 4. *Longitudinal section through a pycnidium showing the pycnidial wall and spores borne along the base and sides of the pycnidium.* ×220.

FIG. 5. *Conidia of S. musiva.* ×440.

FIG. 6. *Conidia produced on 4-day-old colonies growing on potato dextrose agar.* ×430.

FIG. 7. *Twenty-day-old colony resulting from the germination of several conidia.* ×1. *Note that the colony is divided into several morphologically distinct sectors.*
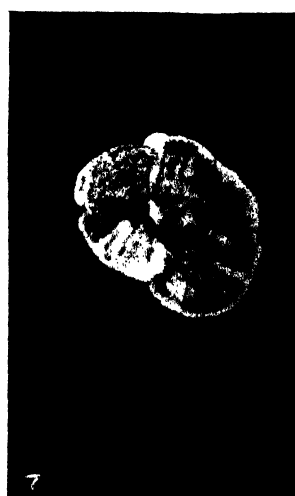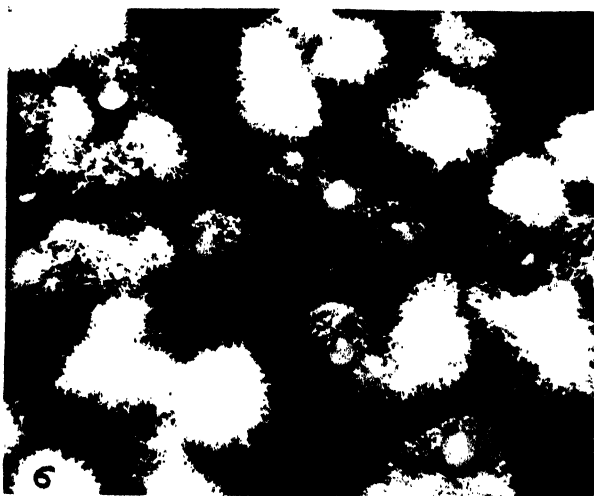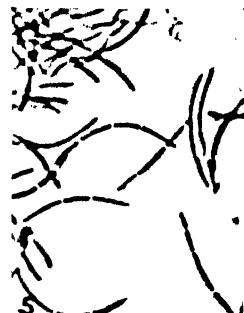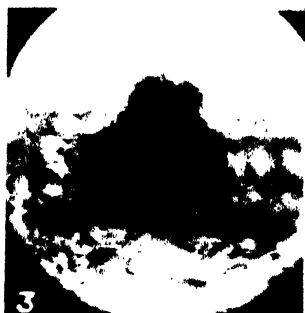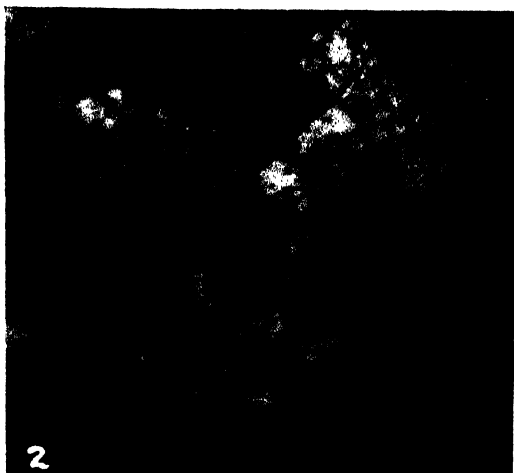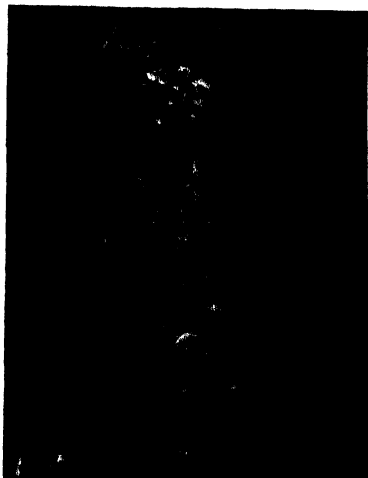
PLATE V

later turning a greenish colour except for the white, advancing margins. After the fourth day, clear to brownish drops of liquid are present on some of the colonies. These soon appear pinkish, filled with conidia from the embedded pycnidia (Plate V, Fig. 6). Mycelial colonies resulting from several spores (ascospores or conidia) germinating and growing together are frequently divided into a number of morphologically distinct sectors (Plate V, Fig. 7). Some colonies do not produce the conidial stage but remain sterile throughout their existence.

Inoculations

During the spring of 1938, inoculations were made under greenhouse conditions on potted cuttings of *P. tacamahaca*, *P. balsamifera*, *P. Rasumowskyana*, *P. Petrowskyana*, *P. berolinensis*, Northwest, and Saskatchewan poplars.

The inoculum was comprised of conidial suspensions in water, the spores having been derived from single ascospore cultures. Parallel series were conducted with inoculum from cottonwood and Russian poplar.

A number of stems were inoculated by puncturing the bark tissues with a sterile needle, and immediately applying a few drops of the inoculum to the incisions (Plate III, Fig. 6). In addition to the above inoculations, spores were painted on unwounded stems and leaves by means of a camel's-hair brush. Checks were established for each inoculation experiment. Both checks and inoculations were incubated for a period of three days, by placing the cuttings under bell-jars standing in trays partly filled with water. The cuttings were then removed and placed upon the greenhouse bench.

RESULTS

The checks of all leaf and stem inoculations remained sterile, and the incisions healed over very rapidly. The inoculum derived from cottonwood leaves produced leaf spots and cankers similar in character to those resulting from the fungus on Russian poplar. Therefore, this evidence, supporting previous morphological and cultural studies, demonstrates that the fungus occurring as a leaf parasite on the native poplar is the same as that producing cankers on Russian, Northwest, and Saskatchewan poplars.

Leaf Spot Stage

From 7 to 21 days after inoculation, leaf lesions, identical with those found in nature, developed on *P. tacamahaca*, *P. balsamifera*, *P. Rasumowskyana*, *P. Petrowskyana*, *P. berolinensis*, Northwest, and Saskatchewan poplars (Plate IV). From two to four weeks after inoculation, pycnidia became evident. Conidia were isolated from these fruiting bodies and the resulting mycelial colonies and conidia agreed with the material used for inoculation.

Canker Stage

Inoculations on wounded stems. Two weeks after inoculation, small brownish to black areas of discoloured bark were visible around the wounds (Plate III, Fig. 6). It is important to note that these lesions were produced on the indigenous species (balsam poplar and cottonwood) in the same manner as on

the Russian and native hybrid poplars. However, a decided difference was observed in the later development of the cankers. Usually within one month the lesions on balsam poplar and cottonwood were delimited by secondary periderm tissue of the hosts, and no further development of the cankers ensued (Plate III, Fig. 4). The lesions on the Russian and native hybrid poplars continued to grow, and frequently girdled the cuttings as early as two months after inoculation. The induced cankers with their black, advancing margins enclosing paler areas with pycnidia were similar in every way to those found in nature (Plate III, Fig. 7). A general summary of the wound inoculations is presented in Table II.

TABLE II

SUMMARY OF STEM WOUND INOCULATIONS MADE DURING MAY AND JUNE, 1938

Host	No. of cuttings inoculated	Canker condition on October 10, 1938			
		No. girdled	Per cent girdled	No. living but with actively growing cankers present	No. with lesions apparently completely healed over
P. Rasumowskyana	21	17	81	4	0
Northwest poplar	15	12	80	3	0
P. Petrowskyana	13	6	46	7	0
P. berolinensis	6	1	17	3	2
Saskatchewan poplar	16	4	25	8	4
P. tacamahaca	16	0	0	2	14
P. balsamifera	24	0	0	0	24

From Table II it is evident that 80% of the cuttings of *P. Rasumowskyana* and Northwest poplar were girdled five months after inoculation. A significant decrease is apparent in the percentage girdled of *P. Petrowskyana*, *P. berolinensis*, and Saskatchewan poplar. No cuttings of the native poplars were killed, although two lesions on balsam poplar appeared active on October 10. The difference in the percentage girdled of the various hosts, supported by the observation that initially small cankers are formed on balsam poplar and cottonwood, suggest that it is not a question of absolute immunity or susceptibility of the hosts to the canker stage. It is, apparently, rather a problem of relative resistance, ranging from *P. Rasumowskyana* and Northwest poplar which may be classed as very susceptible, to *P. Petrowskyana*, *P. berolinensis*, and Saskatchewan poplar which may be considered moderately susceptible, and finally to *P. balsamifera* and *P. tacamahaca* which appear very resistant.

Inoculations on unwounded stems. From 15 to 20 days after inoculation, lesions developed on unwounded stems and leaf petioles of *P. Rasumowskyana*, *P. Petrowskyana*, *P. berolinensis*, Northwest, and Saskatchewan poplars. This type of infection was not observed on *P. tacamahaca* or *P. balsamifera*. The stem cankers were produced at the bases of leaves and surrounding lenticels.

The first symptom of lenticel infection consisted of a swelling of the bark tissues around the lenticels (Plate III, Fig. 5). Almost immediately, elongate, oval, black areas of diseased bark were produced, (Plate III, Fig. 3), which rapidly increased in size and ultimately girdled the stems. Frequently, a number of lenticels occurring close together would become infected and produce a local swelling of the entire stem (Plate III, Fig. 5). These infec-tions usually girdled the cuttings within two weeks following the first appear-ance of the symptoms of disease.

A number of cuttings were inoculated by placing conidia at approximately the mid-point along the leaf petioles. Petiole lesions resulted and the injury progressed down the petioles into the stems with the final production of stem cankers (Plate III, Fig. 1). In other instances conidia were applied to the green stipules at leaf nodes. Lesions with pycnidia were often produced on the stipules and stem cankers developed from the stipule scars (Plate III, Fig. 2).

Re-isolations were made from the infected bark and the conidia produced in cankers resulting from inoculations. The mycelial colonies and conidia obtained were of the same character as the ascospore cultures used as the inoculum.

Discussion

Broadly speaking, two distinct types of problems are encountered in the study of fungous diseases of trees. The one includes the diseases of native trees caused by native organisms, while the other has to do with the destruc-tion of indigenous trees by introduced parasites. The results of this investiga-tion suggest a third type which may be described as the diseases of new hosts, exotic species and hybrids, resulting from the attack of native organisms.

There is reason to believe that in the distant past indigenous trees have acquired a certain degree of resistance to native parasites, and for this reason tend to survive the diseases caused by them. The control of such diseases, therefore, will depend upon the stage of silvicultural development practised in the country.

In this study evidence is presented to demonstrate that *Septoria musiva*, a North American fungus, acts as a virulent canker-producing, as well as leaf-spotting, parasite on Russian, Northwest, and Saskatchewan poplars. The pathogen is widely distributed in Canada and occurs commonly, causing relatively unimportant leaf injury to several of the native species. This organism, therefore, is an excellent example of a native fungus, generally believed of minor importance, becoming an aggressive parasite on new hosts.

Considering the introduced trees (Russian poplars), it is apparent that the universal occurrence of the pathogen provides a continual threat to their culture, thus making control of the disease under field conditions most difficult and uncertain. This fact, in addition to widespread reports of the destruction of Russian poplars by the disease, suggests that the propagation of these species be discontinued in favour of more resistant strains.

Present evidence indicates that Northwest and Saskatchewan poplars have arisen as natural crosses between balsam poplar and cottonwood. Further, their greater ease of propagation by cuttings, more rapid growth, and hardiness point to the conclusion that they exhibit hybrid vigour. Assuming that this explanation of the origin of the hybrids is correct, it is evident that, although they possess more desirable growth characters, they are, nevertheless, susceptible to a new and serious form of disease to which the parent strains are highly resistant. It is not to be inferred that all hybrids between balsam poplar and cottonwood will be equally susceptible to the canker stage. Indeed, inoculation experiments indicated that the Northwest form was more severely attacked than Saskatchewan poplar. Therefore, if the progeny of an artificial cross between balsam poplar and cottonwood were carefully selected for hybrid vigour and disease resistance, it is quite possible that a form would be derived that possessed the growth characters of Northwest and Saskatchewan poplar, and the resistance of the parent trees.

However, it is essential that tree-breeders, and foresters favouring the importation of exotic species, take full cognizance of the possibility that each new variety may serve as a favourable host for organisms that in the past have been recognized as saprophytes or parasites of minor importance. This may result in the development of a new or more serious form of a long established disease, causing severe damage to the new host strain.

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NOTES ON MEALY BUG INJURY ON STRAWBERRY AND ITS RESEMBLANCE TO CRINKLE[1]

BY A. A. HILDEBRAND[2]

Abstract

Mealy bugs, *Pseudococcus* sp., (or spp.) have been shown to be primarily responsible for symptoms which for a number of years have been appearing on strawberry plants grown in the greenhouse. and which in certain respects bear strong resemblance to those of plants affected with the virus disease, Crinkle. The symptoms of the two troubles show similarity, not only in the occurrence on younger leaves of small, circular to irregularly-shaped translucent spots with more intensely chlorotic central portions, but also in the unevenly chlorotic character and malformation of older leaves and in the ultimate general dwarfing of heavily infested plants.

Introduction

In the course of investigations of the pathology of the strawberry during the past few years (3, 4, 5) large populations of plants have been kept under close observation in the greenhouse. As the studies have progressed, it has gradually become possible to recognize the different symptom-pictures associated with various troubles, whether of abiotic origin or the result of attack by parasitic organisms, infection by virus or infestation by certain insect pests. However, the real cause of a particular trouble which has been appearing intermittently for several years on greenhouse plants and which, tentatively, had been diagnosed as Crinkle, was determined definitely only during the past summer. The findings in connection with this particular phase of investigation are embodied in the present report.

Symptomatological Observations

Symptoms first become apparent on the young leaves of affected plants. Such leaves show on their upper surface translucent spots which may be circular, ovoid or angular in shape and which, though very small at first, usually increase in size with leaf expansion (Fig. 3, Fig. 4, A). The spots may occur along a vein or may occupy an intercostal position on the laminae of the leaflet. They may be few in number or very numerous, in which case, often becoming confluent, they produce a mottling so marked that it resembles

a mosaic (Fig. 3). Many of the translucent spots show a minute, more intensely chlorotic central portion, which more usually remains as such but occasionally may become reddish- or brownish-discoloured and then necrotic. If the chlorotic spots appear on all the young leaves as they are formed successively, then up to a certain age all the leaves of an affected plant may show the symptoms. As leaves mature, however, the spots tend to become
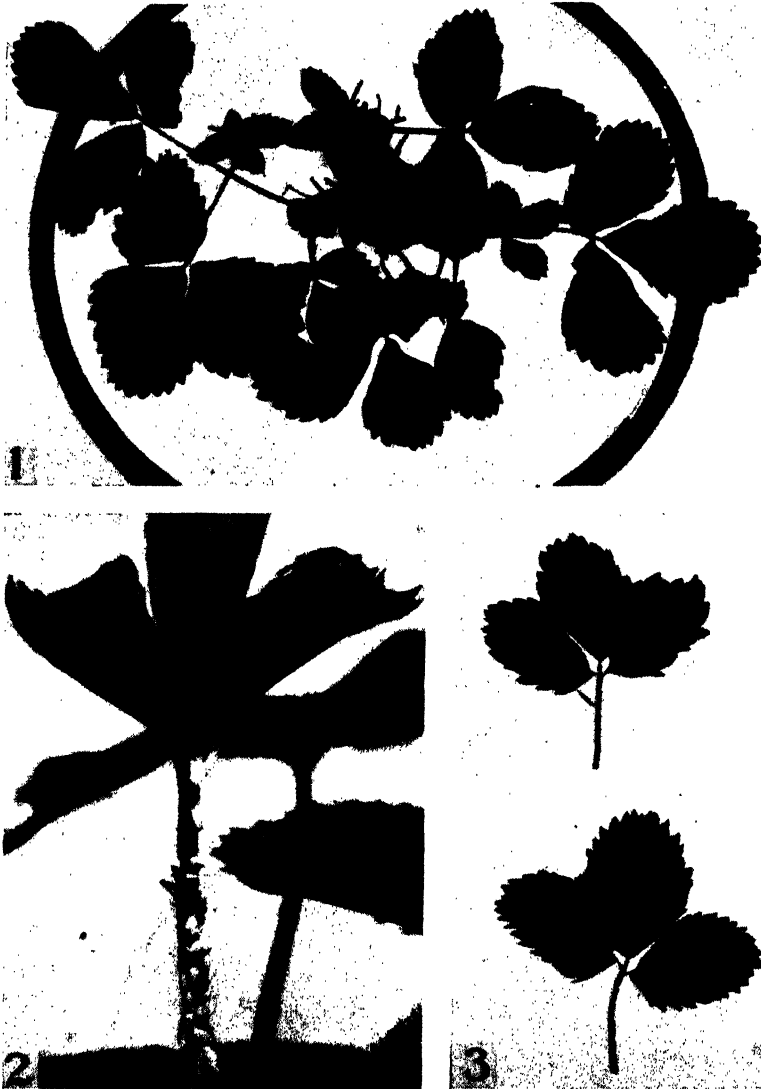
FIG. 1. *Premier plant showing in addition to mottling, chlorosis, dwarfing and mal-formation of leaves, which finally result from heavy, continuous infestation by mealy bugs.*

FIG. 2. *Mealy bugs feeding on petiole of leaf of artificially-infested Blakemore plant.*

FIG. 3. *Leaves detached from artificially-infested Premier plant, showing mosaic-like effect resulting from coalescence of chlorotic areas caused by feeding punctures.*

indistinct and if, as sometimes happens, they fail to appear on young, later-formed leaves, a plant that formerly showed the symptoms may appear perfectly healthy and normal again. In plants which have been affected for some time, the leaves often show, in addition to the spots, marked dwarfing and considerable malformation, accompanied by varying degrees of chlorosis (Fig. 1), but such leaves rarely exhibit on their surface any suggestion of rugosity or crinkling. Many severely affected plants eventually die.

FIG. 4. *Similarity between symptoms of mealy bug injury and Crinkle. A. Two-month-old Fragaria virginiana plant showing symptoms that developed within one month after transfer of mealy bugs, the translucent, chlorotic areas being the result of feeding punctures made by the insects prior to the leaf's full expansion from the bud. B. Runner plant potted while still attached to mother having Crinkle. Symptoms on first leaves are severe; when well-rooted, a leaf (largest) was produced without symptoms, but the youngest leaf showed primary symptoms as the plant became pot-bound. (B, After Zeller, Ore. Exp. Sta. Bull. 319).*

Etiology

(a) Circumstantial evidence—In the spring and early summer of 1938, the number of plants exhibiting the symptoms described above increased so greatly that it became imperative to investigate the condition, the more so because the trouble, occurring as it did among plants which were being used in virus investigations, so complicated the symptom-picture on these plants that it became quite impossible to make correct diagnoses.   Upon careful examination of an affected plant it was found that small colonies of mealy bugs were present among the closely imbricated leaf bases, encircling the crown near the ground level.   The examination of a few additional affected plants showed that mealy bugs were also present on them, or that these insects had been present, as was indicated by accumulations of "skin casts" and the remains of the white cottony sack in which the insect deposits its eggs.   In some instances the insects were quite readily observable on leaf petioles or flower peduncles, whereas in others they occupied more inconspicuous positions among the leaf bases or within the folds of young developing leaves.   With the discovery of the insects it was recalled that Carter (1, 2) has shown that a species of mealy bug, *Pseudococcus brevipes*, causes a spotting of pineapple leaves in Hawaii, illustrations of which (2, pp. 244, 245) suggest a very close similarity to the type of injury observed on the strawberry.   On June 9, 1938, a survey was made of 662 strawberry plants, which at the time constituted the total population of plants in the compartment of the greenhouse where the trouble was confined.   The results are shown in Table I.

TABLE I

MEALY BUG INFESTATION IN RELATION TO OCCURRENCE OF MOTTLED FOLIAGE ON
STRAWBERRY PLANTS IN THE GREENHOUSE

Condition of plants	Total	Mealy bugs			
		Present		Absent	
		No.	Per cent	No.	Per cent
Foliage mottled	120	100	83.3	20	16.7
Healthy	542	24	4.5	518	95.5

The high percentage of plants showing mottled leaves with mealy bugs present furnished strong circumstantial evidence of a correlation between the two.   In the course of the examination it was noted that although individual plants showing mottled foliage might be found scattered among healthy ones, nevertheless, the majority of affected plants occurred in groups.   It appeared as though certain more severely affected plants in these groups might have been the original source from which the insects spread to surrounding plants.

(b) Experimental proof—There were available in June, 1938, in another compartment of the greenhouse, young, healthy plants of the commercial varieties, Premier and Blakemore, and of the wild species *Fragaria virginiana*. Mealy bugs* were transferred to each of 20 plants in a group comprising 8 Blakemore, 8 Premier, and 6 *F. virginiana*, by brushing the insects from an infested leaf or flower stalk held above the healthy plant. It was noted that when the insects happened to alight on the upper surface of a leaf, they crawled immediately to the juncture of the leaflets with the petiole, where some of them remained, while others migrated down the petiole and were lost from sight among the leaf bases and young leaves emerging from the crown. Later, the insects reappeared and were observed along the petioles, where they fed in large numbers (Fig. 2). On all 20 plants to which the insects had been transferred, typical symptoms appeared, but only on the very young leaves that unfolded after the transfer had been made. On none of these plants did the translucent spots ever appear on the laminae of the older, fully-expanded leaflets. Symptoms were observed as early as four days after the transfer of insects. The two commercial varieties and the wild species were apparently equally susceptible to infestation. It was found that the time required for the symptoms to appear depended on the developmental stage of the young leaves. If a leaf were ready to unfold within a few days after the transfer, the symptoms became apparent sooner on that leaf than on others that unfolded later.

All of 10 check plants to which no insects had been added, and which were segregated in another part of the greenhouse, remained healthy.

On July 15, the 20 plants on which the symptoms had developed were divided into two equal groups, each comprising 4 Blakemore, 4 Premier and 2 *F. virginiana*. In one group the mealy bugs were left to feed and multiply. The other group of plants, after fumigation with Cyanogas, were syringed with water under pressure until they were free from the insects. Towards the end of August, the plants comprising the latter group showed no evidence of symptoms and could be regarded only as normal, healthy plants. In the untreated group, however, two plants were dead and the remaining eight showed not only the mottled foliage, but also the dwarfing and chlorosis that follow continuous, heavy infestation.

Although the above experiments had proved almost conclusively that mealy bugs were the cause of the particular trouble under investigation, the question still remained unanswered as to whether the translucent spots on the leaves resulted directly from injury caused by feeding punctures or indirectly from systemic infection following injection into the plant of some toxic or other principle during the feeding process. Carter (1) found that following only a short period of feeding by *Pseudococcus brevipes* on the pineapple, there is a rapid diffusion of a toxic principle, which in approximately two months gives rise to a wilt that involves part or all of a plant. In an attempt to find if some similar phenomenon might be associated with the feeding of mealy bugs on

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* *No attempt has been made to identify the insects beyond the genus and it is possible that more than one species may have been involved in the transfers.*

the strawberry, a second series of experiments was carried out in which the feeding of the insects was more precisely restricted than in the first experiment. By means of Van Tieghem cells, mealy bugs were confined to limited areas on the upper and lower surface of leaflets varying in age from very young to those fully mature. In other cases, the feeding of the insects was restricted to petioles alone. It was found that even though large numbers of insects might feed on the petiole of a leaf, mottling never appeared on the laminae of that leaf, thus proving that the translucent spots are not the result of systemic infection. Spots appeared only in those cases in which the insects had been confined to the under surface of recently unfolded leaves. These findings, together with the fact that during the whole period of investigation insects have never been observed feeding on the laminae of older leaves, indicate that the translucent spots are directly the result of punctures made when the insect is feeding within the folds of the very young leaf.

Symptoms of Mealy Bug Injury Compared with Those of Crinkle

As briefly intimated in the introductory paragraph, the trouble with which the present report is concerned had been diagnosed as Crinkle until the discovery of its true cause. Crinkle is a virus disease which has never been found in Ontario, but has, nevertheless, world-wide distribution. Its occurrence has been reported from the Pacific Northwest States of the United States (8, 9), from England (6) and from Australia (7). It is, perhaps, in the occurrence of translucent or chlorotic spots on the leaves that the symptoms of the two troubles most closely approach one another and in certain cases overlap (Fig. 4, A and B). In both, these chlorotic spots first become apparent on young unfolding leaves as extremely localized pin-point areas, which enlarge with leaf expansion and produce a stippling in Crinkle; whereas in mealy bug injury the effect is rather a mottle or even a mosaic, if a number of spots coalesce. In both troubles, minute, more intensely chlorotic dots appear in the centre of the chlorotic areas. In mealy bug injury these extremely yellowed centres tend to remain as such, but generally in Crinkle they turn reddish or purplish and then, drying out, become necrotic. A few such instances have been observed in mealy bug injury, but they are the exception rather than the rule. As in Crinkle, leaves of plants that have been heavily infested with mealy bugs for a considerable time may show most uneven chlorosis. In mealy bug injury, clearing of veins, which is often characteristic of plants infected with Crinkle, has not been observed. Also as in Crinkle, plants heavily infested with the insects lose most of their erect growth, the plants presenting a flattened appearance as the result of dwarfing of the leaves and shortening of the petioles. Although on heavily infested plants the leaves may be variously distorted as to shape, the surface of such leaves does not present the crinkled or rugose condition that is an important diagnostic symptom of Crinkle. As young affected leaves grow older, they may show a mottling so severe as to be almost mistakable for a mosaic, yet many such leaves otherwise show no departure from the normal.

Discussion

Though a careful search was maintained throughout the past growing season, no mealy bugs could be found on strawberries growing under outdoor conditions. As far as the writer is aware, there is no reference to them in the literature dealing with insect pests of the strawberry. Obviously they are not a factor of economic importance in the production of the crop. From the strictly practical viewpoint, little significance attaches to the discovery of the insects infesting and injuring plants in the greenhouse, the more so since few or no strawberries are grown commercially under glass. But the present report is not without significance otherwise. At the present time the pathology of the strawberry is engaging the attention of research workers in many different centres. Certain phases of investigation extend over considerable periods of time and have to be carried out in the greenhouse, with the use of large numbers of plants. Mealy bugs are a common greenhouse pest. As in the experience of the present writer, an unsuspected infestation of experimental plants may result in a composite symptom-picture, confusing and misleading enough to result in possible error in diagnosis. This may be especially true in the case of plants that show symptoms, but upon which no insects can be found at the time of examination. Referring again to Table I, it will be noted that no insects were found on 20 of the plants included among 120 that showed mottled foliage. It is thought that the present report may be of some value in aiding workers elsewhere to distinguish, early in their investigations, between symptoms that, though almost identical in certain respects, are due to entirely different causes.

Acknowledgments

The writer wishes to express his appreciation to Dr. G. H. Berkeley for continued interest and helpful suggestions during the course of the investigation. Thanks are also due to Dr. S. M. Zeller of the Oregon State Agricultural College for permission kindly granted to reproduce an illustration from his published works.

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NITRIFICATION UNDER AND AFTER ALFALFA, BROME, TIMOTHY, AND WESTERN RYE GRASS

I. NITROGEN ABSORPTION OF HAY CROPS AND SUCCEEDING WHEAT CROPS[1]

By R. Newton[2], R. S. Young[3], and J. G. Malloch[4]

Abstract

The mean annual nitrogen absorption (lb./acre) of hay crops (excluding roots and stubble) was: alfalfa, 94.9; timothy, 39.5; western rye, 62.9; brome, 61.1. For entire hay plants to plow depth, based on sods one and three years old, the corresponding figures were: 241.8, 152.2, 137.8, 154.2. For entire wheat plants following one-, three-, and five-year-old sods of these hay crops for six, four, and two successive years, respectively, the mean values were: 63.4, 58.6, 56.3, 51.9. These figures are taken to indicate roughly the relative rates of soil nitrification under and after the crops in question, except under alfalfa, a legume.

The roots and stubble of the hay plants contained about 71% of the dry matter and 68% of the nitrogen of these plants, whereas the wheat roots and stubble contained only 19% of the dry matter and 9% of the nitrogen of these plants.

Preceding crops and seasonal conditions, especially the latter, affected the protein content and hardness of the wheat grain. Protein content of grain was generally, though not invariably, in the same relative order as indicated rates of soil nitrification after the four hay crops. Protein content was related quantitatively to hardness of grain and to loaf volume of bread, but there was no evidence of qualitative differences in the protein following the various hay crops.

Introduction

The rate at which nitrogen is made available by decomposition of crop residues is a prime factor in crop sequence effects. The measurement of nitrification rates by soil analyses is complicated, in the presence of growing crops, by the rapid absorption of available nitrogen by such crops. After unsatisfactory attempts to relate the yield and nitrogen content of wheat varieties grown in different environments, to the nitrate nitrogen found by periodic sampling and analysis of the soil, the senior author laid down at Edmonton in 1927, in the experimental field of the Department of Field Crops, an experiment designed to overcome the difficulty by using the wheat plants themselves

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PLATE I

PLATE I. General view of experimental block in year of seeding. Photographed on September 2, 1927.

as indicators of nitrification rates. Members of the Department of Soils co-operated by making parallel soil analyses and counts of various classes of soil micro-organisms, in order to have the final picture as complete as possible. The experiment was continued eight years, ending with 1934. During this period several workers collaborated in the investigation of various aspects to be reported now in a series of papers.

The choice of crops was dictated by their importance in Western agriculture and by interests arising out of earlier investigations. The beneficial effects of alfalfa (*Medicago sativa* L.) and other legumes in crop rotations are well known. On the other hand, it has frequently been observed that timothy (*Phleum pratense* L.) exerts a depressive effect on a succeeding grain crop, a subject discussed in detail by Newton and Ficht (2). The action of brome grass (*Bromus inermis* Leyss.) and western rye grass (*Agropyron tenerum* Vasey) were not as well known, though the information available suggested that they fell between the legumes and timothy in this respect. Some results bearing on the point are included in the paper just cited. In another experiment at Edmonton, barley grown the next season after timothy gave plants on the average 37 in. tall, yielding 36.8 bu. per acre of grain containing 13.8% protein; and after western rye grass, plants 41 in. tall, yielding 46.0 bu. per acre, with 15% protein. The crop after western rye grass was thus substantially larger and richer in nitrogen than after timothy.

The generally accepted explanation for the depressive influence of timothy on succeeding crops is the temporary immobilization of soil nitrates by assimilation in the bodies of micro-organisms engaged in the destruction of carbonaceous material in the timothy residues, that is, in the roots and stubble plowed under when the sod is broken. The wider the C : N ratio in crop residues, other things being equal, the greater and more prolonged should be the dearth of nitrates available to the succeeding crop. Accordingly, the sampling and analysis of crop residues were made a part of the present experimental plan.

The hay crops were seeded June 10 to 21, 1927, without a nurse crop, on a block of land that had been summerfallowed the previous season. The block was divided into three sub-blocks, each laid out as a Latin square of 16 plots, each plot 50 by 50 links in size. There being four crops, each occurred four times in each sub-block. An excellent, uniform stand was obtained, as may be judged by Plate I, a photograph taken on September 2, 1927.

Sub-block I was broken on July 23, 1928, just after the hay crop was removed. The sod was thus a little over one year old. Sub-block II was broken at the corresponding time in 1930 and Sub-block III in 1932, these sods being approximately three and five years old respectively. In all cases, Marquis wheat was planted the season after breaking, and each season thereafter until 1934. The number of successive wheat crops was thus six, four and two, on Sub-blocks I, II, and III, respectively.

The following tabular summary of crop sequence will remind the reader of the history of the three sub-blocks, in the course of the presentation and discussion of results.

	I	II	III
1926	Fallow	Fallow	Fallow
1927	Seeded	Seeded	Seeded
1928	Hay	Hay	Hay
1929	Wheat	Hay	Hay
1930	Wheat	Hay	Hay
1931	Wheat	Wheat	Hay
1932	Wheat	Wheat	Hay
1933	Wheat	Wheat	Wheat
1934	Wheat	Wheat	Wheat

Since moisture exercises a dominant influence on both rate of nitrification and crop growth, it will be useful to give here also the inches of precipitation at Edmonton for the seasons of this experiment. With reference to the wheat crops it must be borne in mind that August rainfall has only a limited effect on the current season's growth, while subsequent autumn rainfall must be considered wholly in relation to the next season's crop. May-July rainfall is of course especially important. During this period it was substantially in defect in 1929 and 1930, and moderately so in 1932.

	April	May	June	July	August	September	October
1926	0.32	3.61	3.25	2.21	4.84	0.98	1.29
1927	0.61	2.03	3.10	4.25	0.81	2.79	0.58
1928	0.92	2.14	2.17	4.94	1.70	2.14	0.26
1929	1.53	0.65	1.59	3.79	2.27	0.71	0.31
1930	0.62	1.77	2.59	1.88	1.44	0.34	0.14
1931	0.45	1.80	5.97	3.06	4.20	1.25	0.73
1932	2.03	1.63	2.09	3.33	0.52	0.56	1.00
1933	0.72	1.98	3.35	3.70	1.43	0.98	0.51
1934	1.46	2.86	4.18	2.97	1.72	1.86	1.78

Unfortunately, an invasion of "take-all" disease caused by *Ophiobolus graminis*, beginning in 1931 and increasing in severity thereafter, ruined the western rye plots of Sub-block III and damaged to some extent the wheat plots following that grass on Sub-block II, with some injury also to various other plots, especially to wheat following alfalfa in 1932 on Sub-block II. This factor contributed to the unavoidable error of the experiment.

As usually happens in a long continued and rather extensive experiment, a number of side issues became the objects of minor investigations, the more interesting results of which have been made the subjects of certain papers in this series. This first paper is concerned with nitrification as measured by

the yield and nitrogen content of the crops and residues, with incidental reference to effects on the baking quality of the wheat. The completeness of the absorption of available nitrogen by the growing crop will be shown in data to be published in Part II of the series.

Sampling and Analyses

Yields of both hay and wheat were determined on the crop harvested after first trimming the 50-link square plots to 46 links square, in order to minimize border effects. On two opposite sides, trimming meant simply removing two rows, since the plots were seeded in drills one link apart.

Samples were taken of each cutting of hay, and of the sods at the time of breaking. The wheat crops were sampled with respect to grain, straw, stubble, and roots to a depth of 6 in. (plow depth). Each plot was sampled individually, in the following quantities: hay, 1000 gm. fresh weight; sods, four per plot, each ½ sq. ft. in area and 6 in. deep; straw, 1000 gm. (at threshing); grain, 2000 gm.; stubble and roots, same as for hay sods. The sods were washed out promptly, to avoid fermentation, and roots and stubble carefully divided at the crown. Fresh material was dried rapidly in a drier with a strong current of air at about 65° C. Samples of vegetative parts were allowed to come to air-dry equilibrium, then re-weighed, ground in a Wiley mill and re-sampled for final determination of moisture in a vacuum oven at 98° C. and of nitrogen by a modified Kjeldahl method. Further analyses of the hay residues will be reported in a later paper of this series. The wheat samples were milled and baked, with associated tests of grade, weight per bushel, protein content and kernel texture.

In most cases, individual plot samples were carried through the analyses separately, to secure an estimate of variability. The residues, however, after being dried and weighed, were combined into composite samples for each hay crop in each sub-block, and the hay samples themselves were combined in this way in certain years, as the variation in nitrogen content of the individual plot samples was small. Wheat stubble and roots were not sampled in 1929, and in that season, too, the replicate plot samples of wheat, after the recording of individual plot yields, were combined before analysis.

Yield and Nitrogen Absorption of Hay Crops

While the main purpose of the experiment was to measure the rate of soil nitrification as affected by the hay crop residues, it seems worth while to indicate the relative draft on soil nitrogen made by the removal of the hay crops, since this may have affected the subsequent growth of the wheat crops used as indicators of nitrification. The data covering this point are given in Table I and Fig. 1. The order in which the hay crops are listed in Table I and later tables is that of the absorption of nitrogen by succeeding wheat crops (Table VII and Fig. 1), this being the crucial point of the experiment.

In the year of seeding, only one cutting of hay was taken from each sub-block, and in the year of breaking only one cutting from the particular sub-

block concerned.   In the dry season of 1929 there was not enough growth of timothy to make possible a second cutting of this crop.   In 1932 no crop of western rye was secured from Sub-block III, the grass having been killed by the "take-all" disease.   In the remaining cases, two cuttings were made of each hay crop each season.   The very low yields of timothy in 1929 and 1930 are due to its susceptibility to drought, these being seasons of sub-normal rainfall.   The draft upon soil nitrogen made by this crop in those seasons was correspondingly small.

TABLE I

YIELD AND NITROGEN ABSORPTION OF HAY CROPS

Sub-block	Year	Hay yield, dry basis, lb. per acre				Nitrogen absorbed, lb. per acre				Nec. diff. at 5% level
		Alfalfa	Timothy	Western rye	Brome	Alfalfa	Timothy	Western rye	Brome	
I	1927	1217	2611	2587	2044	43.7	53.2	68.5	70.9	
	1928	4041	4773	5825	5269	113.4	66.2	85.1	108.7	
	Mean	2629	3692	4206	3656	78.5	59.7	76.8	89.8	4.6
II	1927	1170	2422	2587	1855	42.5	43.7	60.3	65.0	
	1928	6345	5624	7916	7290	155.9	81.6	127.6	132.3	
	1929	4112	343	2564	1760	116.0	7.0	36.2	25.0	
	1930	2091	319	1666	1205	53.0	4.0	24.0	17.0	
	Mean	3430	2177	3683	3028	91.8	34.1	62.0	59.8	4.8
III	1927	1418	2800	2658	1749	53.2	55.5	63.8	63.8	
	1928	6097	6073	7786	7313	150.0	85.0	124.0	126.4	
	1929	3840	319	1855	1630	105.0	6.0	55.6	29.2	
	1930	3521	496	1453	1146	92.0	6.4	22.1	15.0	
	1931	4679	1879	1607	1170	119.0	26.0	25.0	19.0	
	1932	3403	2292	–	1134	73.0	19.0	–	15.0	
	Mean*	3911	2313	3072	2602	103.8	35.8	58.1	50.7	2.1
General mean*		3503	2514	3500	2948	94.9	39.5	62.9	61.1	

* *Means calculated without 1932 results for Sub-block III, because of failure of western rye.*

Inspection of Table I shows that alfalfa made less growth than the grasses in the year of seeding, but thereafter maintained its yield better than the grasses, and in general mean yield was equalled only by western rye (neglecting 1932, when western rye was killed by "take-all").   In absorption of nitrogen, alfalfa greatly exceeded the grasses in general mean, but its actual draft on the soil may well have been less than that of the grasses, owing to its symbiotic use of atmospheric nitrogen.   Among the grasses the order of both mean yield and nitrogen absorption of hay in Sub-blocks II and III was western rye, brome, timothy.   In Sub-block I, timothy slightly surpassed brome in yield, but fell significantly below the latter in nitrogen absorption.

The analyses of variance of nitrogen absorbed by the hay crops are given in Table II.   Variations due to soil heterogeneity were moderately significant in one direction, and were differentially affected by season as shown by the interaction of rows with years.   Differences due to crops, to seasons and to interaction of crops with seasons were highly significant.   Reference to the right half of Table I shows that absorption of nitrogen by alfalfa in every sub-block was about three times as great in the second season as in the first, while none of the grasses much more than doubled the first year's absorption.
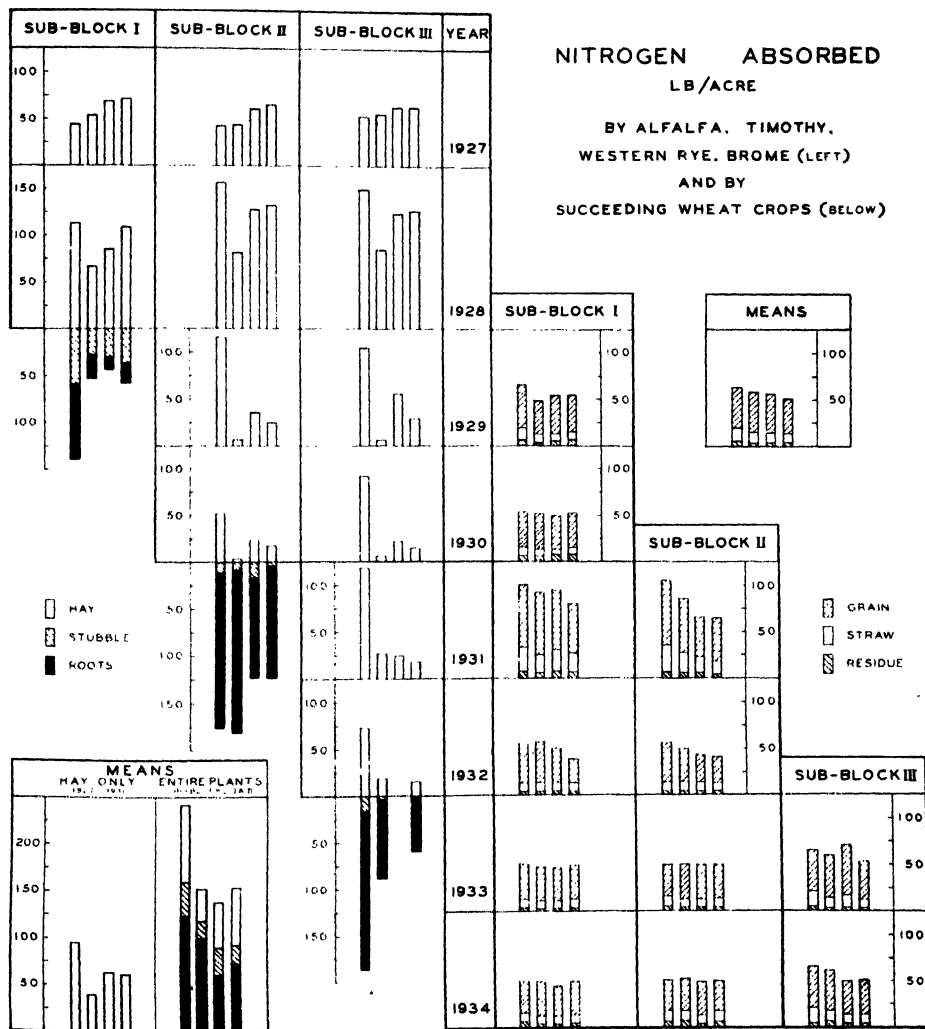
FIG. 1.   *Nitrification as indicated by nitrogen absorption (lb./acre) of successive crops throughout whole course of experiment, 1927–34.   Histograms for alfalfa, timothy, western rye, and brome grass shown from left to right in each group.   That half of figure showing absorption by wheat crops following these sods has been displaced to right to save space.   For same reason (and others given in text) absorption by roots and stubble of hay crops in individual years is shown below the base line of histograms concerned.*

This is a special case resulting from the addition of increased symbiosis to increased growth in the alfalfa. The variances due to crops and years and their interaction were due in general to yield differences, accentuated by differential responses to seasonal moisture supply, or lack of it (compare drought-susceptible timothy with drought-resistant western rye in 1929 and 1930) and by differences in percentage composition of the crops.

TABLE II

ANALYSES OF VARIANCE OF NITROGEN (LB. PER ACRE) ABSORBED BY HAY CROPS

	Variation due to	Sub-block I		Sub-block II		Sub-block III	
		D.f.	Mean square	D.f.	Mean square	D.f.	Mean square
Inter-plot comparisons	Rows	3	1,050.3*	3	498.6	3	286.5*
	Columns	3	477.7	3	282.7	3	47.8
	Crops	3	1,079.2*	3	8,705.9**	3	17,491.3**
	Error (a)	6	134.9	6	183.8	6	43.2
Intra-plot comparisons	Years	1	8,564.1**	3	30,153.6**	4	19,914.3**
	Crops × years	3	1,216.8**	9	2,125.8**	12	2,036.7**
	Rows × years	3	162.7*	9	99.4*	12	57.7
	Columns × years	3	30.5	9	28.7	12	46.3
	Error (b)	6	29.6	18	39.0	24	56.2

* *Exceeds 5% level of significance.*
** *Exceeds 1% level of significance.*

The general mean percentages of nitrogen in the hay are given in the top section of Table IV. Already lowest in hay yield (Table I), timothy falls relatively still lower in nitrogen absorption of hay crop, owing to its low percentage content of this element. Brome, on the other hand, by reason of its higher nitrogen content, achieves practical equality with western rye in nitrogen absorption of hay crop.

Returning to Table II we see, finally, that errors (a) and (b), representing the residual variations of plots within crops, are satisfactorily small.

In Table III are given the yield and nitrogen absorption of the entire hay plants (to plow depth) in the year of breaking each sub-block, these being the only seasons in which the sods were sampled and roots and stubble available for analysis. In this case no analysis of variance for nitrogen absorbed can be made, since as already stated the residues for each crop in each sub-block were combined before analysis. The new information relates to the roots and stubble, the hay yields having been included with those of the other years reported in Table I.

The general advantage that alfalfa possessed in hay yield is offset by the somewhat greater proportional development of roots and stubble in the grasses, the feature that, together with the fineness of their roots, makes grasses particularly valuable in the restoration of "fibre" to soils inclined to drift. This relation is shown on a weight basis in the left half of Table III

and on a percentage basis in the fourth column of Table IV.   In this experiment, timothy excelled the other grasses in weight of residues.   It might be expected that the residues would accumulate as the sods grew older, but this did not happen.   The stubble reached its maximum weight in the one-year-old sods (from which, however, hay cuttings had been taken in both 1927 and 1928) and the roots their maximum in three-year-old sods.   After these maxima, decomposition apparently proceeded at a greater rate than accretion by new growth.

TABLE III

YIELD AND NITROGEN ABSORPTION OF ENTIRE HAY PLANTS

		Yield, dry basis, lb. per acre				Nitrogen absorbed, lb. per acre			
		Alfalfa	Timothy	Western rye	Brome	Alfalfa	Timothy	Western rye	Brome
Sub-block	Hay	4041	4773	5825	5269	113.4	66.2	85.1	108.7
I	Stubble	2338	2954	3199	2790	59.0	27.0	30.1	36.0
1928	Roots	3977	2877	994	2266	80.0	26.1	13.0	23.0
	Total	10356	10604	10018	10325	252.4	119.3	128.2	167.7
Sub-block	Hay	2091	319	1666	1205	53.0	4.0	24.0	17.0
II	Stubble	694	938	1571	448	12.4	8.6	16.1	2.8
1930	Roots	7617	10465	6053	9921	165.7	173.1	107.3	120.6
	Total	10402	11722	9290	11574	231.1	185.7	147.4	140.4
Mean total, Sub-blocks I and II		10379	11163	9654	10950	241.8	152.2	137.8	154.2
Sub-block	Hay	3403	2292	–*	1134	73.0	19.0	–*	15.0
III	Stubble	689	628	–	215	16.7	3.4	–	1.5
1932	Roots	7057	7519	–	5122	170.1	85.0	–	57.3
	Total	11149	10439	–	6471	259.8	107.4	–	73.8
Mean total, all sub-blocks		10636	10922	–	9457	247.8	137.5	–	127.3

* Western rye killed by "take-all" disease.

In the histograms in Fig. 1, the nitrogen absorbed by the roots and stubble of the hay crops is shown on the same scale as that of the hay, but below the base line (except in the means) in order not to interfere with the graphic comparison of the hay fractions in all years.   Also, these roots and stubble, as noted above, are not the product of one year's growth, but represent the net gain of growth over decomposition up to the time of breaking.   The wheat roots and stubble, on the other hand, are the product of one season.   They are combined in the graph as "residue", the values being too small to plot separately.   They are plotted above the base line, since they represent an integral part of the nitrification the wheat plants were used to measure.   The numerical values of the fractions plotted are given in the last column of Table IV.   It must be borne in mind that the roots were taken only to plow depth.

TABLE IV

MEAN NITROGEN CONTENT OF VARIOUS CROP FRACTIONS AND MEAN PERCENTAGES OF TOTAL
YIELD AND NITROGEN ABSORPTION REPRESENTED BY SUCH FRACTIONS

Hay plants

Fraction	Crop	N content, %	Percentage of total yield	Percentage of total N absorption
Hay	Timothy	1.58	22.5	21.6
	Western rye	1.73	38.8	39.6
	Brome	1.90	26.8	36.8
	Grass mean	1.74	28.0	31.7
	Alfalfa	2.74	29.9	32.2
Stubble	Timothy	.81	13.8	9.5
	Western rye	1.03	24.7	16.8
	Stubble	.92	12.2	10.6
	Grass mean	.91	15.8	11.7
	Alfalfa	2.30	11.7	11.8
Roots	Timothy	1.27	63.7	68.9
	Western rye	1.62	36.5	43.6
	Brome	1.16	61.0	52.6
	Grass mean	1.32	56.2	56.6
	Alfalfa	2.22	58.4	55.9
Residues (roots + stubble)	Timothy	1.04	77.5	78.4
	Western rye	1.33	61.2	60.4
	Brome	1.04	73.2	63.2
	Grass mean	1.11	72.0	68.3
	Alfalfa	2.26	70.1	67.8

Wheat plants

Fraction	Crop	N content, %	Percentage of total yield	Percentage of total N absorption
Grain		2.74	30.5	70.9
Straw		.48	50.3	20.1
Stubble		.41	13.2	4.6
Roots		.86	5.9	4.5
Residues		.51	19.1	9.1

In spite of the larger proportion of roots and stubble in the grasses, alfalfa maintains its pre-eminence in nitrogen absorption, whether of entire plants or of residues (Table III, right) by reason of the higher percentage of nitrogen in all its fractions (Table IV, Column 3). Of course, only part of this alfalfa nitrogen was taken from the soil. Among the grasses, the order of nitrogen

absorption by the entire plant is, in one-year-old sod: brome, western rye, timothy. This order is reversed in three- and five-year-old sods (neglecting western rye in the last instance). In nitrogen of roots alone, timothy leads the grasses in all cases, its superior yield more than offsetting its lower nitrogen percentage.

A striking feature observable in Tables III and IV and Fig. 1 is the very large proportion of the hay plants which remains as residue in the soil. The quantity of residue left by wheat plants is almost negligible by comparison. Obviously the rate and manner of decomposition of hay residues are important to succeeding crops.

Yield and Nitrogen Absorption of Wheat

As wheat yields have an intrinsic interest, those obtained in this experiment will be noted before considering them as indicators of nitrification. In Table V are shown the yields of grain, straw, residues, and total crop. Roots and stubble were actually determined separately; their mean composition and proportions, together with those of other fractions of the wheat plants, were indicated in the bottom section of Table IV.

The mean yield of grain, which accounted on the average for 30.5% of the dry weight of the plants, was in all three sub-blocks greatest after timothy. In Sub-block I it was lowest after brome. The only other important differences observable in grain yields are in Sub-block III, where the yield after alfalfa was apparently reduced by competition with volunteer alfalfa plants (a common condition when old stands of alfalfa are broken), and the yield after western rye was increased by the partial fallow of these plots in 1932, consequent on the killing of the hay plants by "take-all".

Straw accounted on the average for about half the dry weight of the plants. The order of straw yield in general followed that of grain yield, though there was a distinct tendency for wheat crops following alfalfa to throw a higher proportion of straw, this access of vegetative activity doubtless reflecting the more liberal supply of available nitrogen in these plots.

Roots and stubble together made up less than one-fifth of the dry weight of the plants, and they are treated as a unit in Table V. They were not determined in 1929. With a fraction as small as this, yield differences are of restricted significance. There appears some tendency, however, for residues to make up a larger proportion of the wheat crop after brome grass than after the other hay crops.

In yield of entire plant, wheat after alfalfa supersedes wheat after timothy in Sub-block I, but in the other two sub-blocks wheat after timothy retains the lead it exhibited with respect to yield of grain alone.

For grain yield the variance has been calculated and is given in Table VI. Soil heterogeneity apparently did not play a major part, the mean square being significant only for the columns of plots in Sub-block II. The statistically very significant variance between crops in Sub-block III must be

TABLE V

YIELD OF WHEAT (DRY BASIS, LB. PER ACRE) AFTER ALFALFA AND THREE GRASSES

	Year	Sub-block I Alfalfa	Timothy	Western rye	Brome	Sub-block II Alfalfa	Timothy	Western rye	Brome	Sub-block III Alfalfa	Timothy	Western rye	Brome
Grain	1929	1764	1284	1488	1392								
	1930	1158	1194	1122	1140								
	1931	2352	2280	2304	2172	2196	2220	1656	1692				
	1932	1368	1620	1476	1092	876†	1338	1122	1116				
	1933	1524	1512	1500	1536	1122	1344	1332	1344	1206	1692	1818	1560
	1934	1500	1488	1452	1470	1218	1392	1446	1278	1410	1776	1410	1524
	Mean*	1580	1619	1571	1482	1353	1574	1389	1358	1308	1734	1614	1542
	Nec. diff.	67.4				70.4				51.2			
Straw	1929	1879	1423	1571	1465								
	1930	1394	1394	1285	1276								
	1931	3875	3592	3875	3875	4064	4064	3214	3214				
	1932	2434	2836	2611	2174	1938†	2363	2103	2079				
	1933	2198	1878	2008	2115	1666	1689	1724	1819	2090	2267	2668	2088
	1934	2659	2517	2493	2536	2292	2422	2505	2304	2930	3214	2611	2588
	Mean*	2512	2443	2454	2395	2490	2634	2386	2354	2510	2740	2640	2338
Residues	1929	–	–	–	–								
	1930	789	768	792	859								
	1931	1157	877	1164	1115	898	1028	835	1023				
	1932	682	819	714	726	654†	954	885	977				
	1933	1052	1011	752	1073	916	914	1022	1015	872	999	1087	854
	1934	1295	863	649	1081	1022	1291	786	1112	908	1345	–	1019
	Mean	995	868	814	971	872	1047	882	1032	890	1172	–	936
Totals	1929	–	–	–	–								
	1930	3341	3356	3199	3275								
	1931	7384	6749	7343	7162	7158	7312	5705	5929				
	1932	4484	5275	4801	3992	3468†	4655	4110	4172				
	1933	4774	4401	4260	4724	3704	3947	4078	4178	4168	4958	5573	4502
	1934	5454	4868	4594	5087	4532	5105	4737	4694	5248	6335	–	5131
	Mean	5087	4930	4839	4848	4716	5255	4658	4743	4708	5646	–	4816

* Excluding 1929.

† One of the four replicate plots estimated to be reduced 60% in yield by "take-all".

attributed at least in part to the circumstances already mentioned, namely, the effects of volunteer alfalfa and "take-all". The intra-plot comparisons bring out the very significant influence of season on the yield of grain, exercised differentially on plots following the different sods, as indicated by the interaction of crops and years and illustrated by the comparative yields in 1929 and 1930 after the various sods in Sub-block I (Table V). The diminishing superiority of alfalfa sod with successive cropping to wheat is also reflected in the interaction of crops with years.

TABLE VI

ANALYSES OF VARIANCE OF YIELD OF GRAIN, LB. PER ACRE

	Variation due to	Sub-block I		Sub-block II		Sub-block III	
		D.f.	Mean square	D.f.	Mean square	D.f.	Mean square
Inter-plot comparisons	Rows	3	31,271.2	3	129,230.1	3	46,579.1
	Columns	3	66,138.2	3	281,523.6*	3	6,537.4
	Crops	3	86,292.9	3	176,864.1	3	256,045.1**
	Error (a)	6	54,603.6	6	39,803.8	6	10,524.4
Intra-plot comparisons	Years	5	2,314,738.6**	3	2,085,429.6**	1	12,880.1
	Crops × years	15	62,019.2**	9	148,050.5**	3	140,752.1**
	Rows × years	15	18,319.4	9	28,238.5	3	20,632.1
	Columns × years	15	18,289.0	9	33,698.0	3	1,279.9
	Error (b)	30	9,578.9	18	15,249.3	6	10,501.8

Nitrogen absorption by the wheat crops following the different sods is shown in Table VII and Fig. 1 (right). In Sub-block I, 1929, the residues were not determined, and the values given were calculated from the mean ratio of residue to total plant in the other five years of the experiment. The histograms for that year could not be drawn without resorting to this expedient, which, however, can introduce no serious error in view of the small fraction represented by the residues. Indeed, as already noted, the disparity between the quantities of residue left by the wheat and the hay plants is one of the most striking features of this experiment. Because of their diminutive quantity, the roots and stubble of the wheat plants are not shown separately in Table VII and Fig. 1, but the mean proportions and nitrogen content of each may be found at the bottom of Table IV. In this table we see that the roots were more than twice as rich in nitrogen as the stubble. The latter did not differ markedly from the straw in this respect. The mean values in the upper half of Table VII show that, while much the largest quantities of nitrogen are fixed in the grain, the relative differences between crops following alfalfa and the grasses are greater in the straw.

While the histograms in the right-hand side of Fig. 1 suggest that the faster rate of nitrification in decomposing alfalfa sods may last only two seasons after breaking, though with some indication of superiority over the grass sods in the third and fourth seasons in Sub-block I, there is other evidence of persistent differences in the character of growth following alfalfa and the grasses. In

TABLE VII

NITROGEN (LB. PER ACRE) ABSORBED BY WHEAT CROPS AFTER ALFALFA AND GRASSES

	Year	Sub-block I				Sub-block II				Sub-block III			
		Alfalfa	Timothy	Western rye	Brome	Alfalfa	Timothy	Western rye	Brome	Alfalfa	Timothy	Western rye	Brome
Grain	1929	47.7*	36.4	43.0	39.3	69.2	58.6	44.5	47.2				
	1930	39.0	39.2	36.9	37.3	26.0†	35.8	30.1	27.8				
	1931	66.9	66.6	64.2	53.5	34.6	36.4	37.1	35.8				
	1932	42.8	43.9	38.1	26.5	34.1	35.0	36.1	32.1	44.0	45.0	53.0	40.6
	1933	37.4	36.9	36.5	36.4					44.8	44.5	35.5	37.2
	1934	33.6	32.5	32.2	31.9								
	Mean	44.0	42.1	41.8	37.5	41.0	41.4	37.0	35.7	44.4	44.8	44.2	38.9
Straw	1929	12.3	8.0	8.0	9.0	28.3	20.8	16.2	12.8				
	1930	7.9	7.1	6.6	7.1	10.2†	8.4	7.6	7.3				
	1931	25.6	20.6	22.6	19.2	10.8	8.4	7.7	9.4				
	1932	9.6	10.2	9.5	9.0	11.6	9.9	9.1	11.0	16.6	9.8	12.9	8.5
	1933	9.5	7.8	7.5	8.2					16.2	10.9	9.4	9.3
	1934	9.5	8.4	7.9	8.4								
	Mean	12.4	10.4	10.4	10.2	15.2	12.0	10.2	10.1	16.4	10.4	11.2	8.9
Grain + straw	Mean	57.0	52.4	52.2	47.6	56.2	53.4	47.1	45.9	60.8	55.1	55.4	47.8
	Nec. diff.	1.30				2.66				1.46			
Residues	1929	6.7*	4.1*	5.1*	6.0*	6.3	5.6	5.4	4.7				
	1930	6.6	6.5	7.6	8.1	4.0†	4.8	4.6	4.7				
	1931	7.6	5.2	7.7	6.5	5.2	4.1	4.3	4.6				
	1932	4.5	4.5	4.5	4.8	5.5	6.2	3.3	5.5	5.1	3.3	4.6	3.3
	1933	4.6	3.9	3.1	4.1					4.8	5.9	4.5*	4.5
	1934	6.3	3.6	2.6	4.8								
	Mean	6.0	4.6	5.1	5.7	5.2	5.2	4.4	4.9	5.0	4.6	4.6	3.9
Totals	1929	66.7	48.5	56.1	54.3	103.8	85.0	66.1	64.7				
	1930	53.5	52.8	51.1	52.5	40.2†	49.4	42.3	39.8				
	1931	100.1	92.4	94.5	79.1	50.6	48.9	49.1	49.8				
	1932	56.9	58.6	52.1	40.3	51.2	51.1	48.5	48.6	65.7	58.1	70.5	52.4
	1933	51.5	48.6	47.1	48.7					65.8	61.3	49.4	51.0
	1934	49.4	44.5	42.7	45.1								
	Mean	63.0	57.6	57.3	53.3	61.4	58.6	51.5	50.7	65.8	59.7	60.0	51.7

* Calculated.

† See footnote to Table V.

Table X we find the wheat grain produced after alfalfa on Sub-block II definitely higher in nitrogen than that produced after the grasses, a superiority reflected in substantially larger loaf volumes, in all four seasons, though the differences in nitrogen absorption by the entire wheat plants after the different sods in the third and fourth seasons are too small to be noticeable in the histograms. Brome seems to be inferior to the other two grasses in its sequence effects on wheat, whether judged by the nitrogen percentage of the kernels (Table X) or the nitrogen absorption by the entire wheat plants (Table VII). The difference between the mean values for the total nitrogen absorption by wheat after timothy and western rye is small except in Sub-block II, where the advantage lies with timothy. Certainly there is in these data no evidence of the supposed deleterious effects of timothy on succeeding crops.

Analyses of variance of nitrogen absorbed by wheat grain and straw combined are given in Table VIII. Residues could hot be included, as they were not analyzed separately for each plot, but they represent on the average only 9% of the total nitrogen absorption. Again we find only moderate differences attributable to soil heterogeneity, the important ones depending upon crops, seasons, and the interaction of these two factors.

TABLE VIII

ANALYSES OF VARIANCE OF NITROGEN (LB. PER ACRE) ABSORBED BY WHEAT GRAIN
AND STRAW

	Variation due to	Sub-block I		Sub-block II		Sub-block III	
		D.f.	Mean square	D.f.	Mean square	D.f.	Mean square
Inter-plot comparisons	Rows	3	25.3	3	153.3	3	61.9*
	Columns	3	63.5	3	93.1	3	9.9
	Crops	3	290.8**	3	531.9**	3	213.8**
	Error (a)	6	16.8	6	60.0	6	8.5
Intra-plot comparisons	Years	4	5,148.5**	3	3,639.7**	1	283.8**
	Crops × years	12	73.5**	9	433.4**	3	203.6**
	Columns × years	12	60.5*	9	45.4*	3	14.3
	Rows × years	12	19.6	9	31.7	3	37.1*
	Error (b)	24	20.3	18	14.4	6	5.7

Quality of Wheat

While a study of the effect of crop sequence on wheat quality was not a prime object of these experiments, the grain produced was subjected to the routine quality tests of the laboratory. No important differences were found in commercial grade, weight per bushel, or flour yield, but the data pertaining to protein content, kernel texture, and loaf volume (bromate baking formula) showed enough variation to be worthy of examination. This is most conveniently accomplished by statistical analyses. In 1929 the grain samples from the four replicates in Sub-block I were combined before analysis, milling and baking. These are therefore excluded from further consideration, except with respect to the mean values recorded in Table X.

Analyses of variance of percentage of protein in wheat, reported in Table IX, show fortuitous variations due to such factors as soil heterogeneity, to be for the most part insignificant.   There are, however, very important differences due to crops and years and their interactions.   The influence of season on protein content is well known, but its differential effect on protein content following various hay crops is of additional interest.   Differences in both quantity and quality of nitrifiable residue left by these crops would affect their potential response to seasonal factors, and so account for the inter-action noted.   Since this comes in question chiefly between the alfalfa and the grasses, it may be suggested that the relative slowness of the alfalfa in starting the first season, and the fact that the sods in Sub-block I were only one year old when broken, explain the lack of significant variance due to crops and their interaction with years in this instance.   Actually, it is clear from Fig. I that more nitrogen was made available to the crop following alfalfa in Sub-block I, especially in 1929 (unfortunately excluded from the cal-culation of variance).

TABLE IX

ANALYSES OF VARIANCE OF PERCENTAGE PROTEIN IN WHEAT (13.5% MOISTURE)

	Variance due to	Sub-block I		Sub-block II		Sub-block III	
		D.f.	Mean square	D.f.	Mean square	D.f.	Mean square
Inter-plot comparisons	Rows	3	.97	3	.20	3	.20
	Columns	3	6.23*	3	3.57	3	.77
	Crops	3	2.97	3	17.97**	3	32.50**
	Error (a)	6	1.13	6	.87	6	.78
Intra-plot comparisons	Years	4	65.63**	3	4.10**	1	16.80**
	Crops × years	12	.33	9	1.61**	3	1.13**
	Rows × years	12	.05	9	.40	3	.10
	Columns × years	12	.33	9	.12	3	.2/*
	Error (b)	24	.29	18	.19	6	.05

Reference to Table X shows that Sub-block I differed from the other two in that the wheat grain was not richer in nitrogen following alfalfa than following the grasses, conspicuously so in 1929, when the plots following alfalfa showed their greatest superiority in total nitrogen supplied to the wheat plants.   Evidently the conditions in these plots were such as to induce vigorous growth and large yields of both straw and grain (Table V), with the nitrogen absorbed thus distributed through more material and the per-centage concentration reduced.   Analytical data on wheat grown under different soil and climatic conditions are, in routine investigations, often limited to nitrogen content of the grain.   Obviously this is not a safe guide to the rate of nitrification in the soil.   Nor does soil richer in available nitrogen necessarily give stronger wheat and larger loaf volumes.   It remains true, however, that the older sods of Sub-blocks II and III invariably produced richer and stronger wheat after alfalfa than after the grasses.   There was

little difference in this respect between timothy and western rye grass, but wheat after brome grass was somewhat inferior in quality, even on the young sod of Sub-block I.

TABLE X

MEANS: LOAF VOLUME (CC.) AND NITROGEN CONTENT OF WHEAT (%, DRY BASIS)

Sub-block	Year	Alfalfa		Timothy		Western rye		Brome	
		Loaf volume	Nitrogen	Loaf volume	Nitrogen	Loaf volume	Nitrogen	Loaf volume	Nitrogen
I	1929	650	3.08	674	3.22	636	3.29	634	3..6
	1930	804	3.33	756	3.28	809	3.31	765	3.27
	1931	779	2.85	754	2.85	711	2.79	651	2.47
	1932	628	2.71	636	2.71	620	2.58	614	2.44
	1933	541	2.46	529	2.44	542	2.44	532	2.39
	1934	423	2.24	433	2.43	460	2.21	423	2.18
II	1931	849	3.15	679	2.64	665	2.73	541	2.20
	1932	700	2.99	600	2.68	621	2.70	602	2.50
	1933	695	3.08	618	2.71	626	2.78	609	2.68
	1934	638	2.80	541	2.51	560	2.50	538	2.50
III	1933	775	3.65	575	2.67	633	2.89	586	2.62
	1934	689	3.18	514	2.51	536	2.52	532	2.44

The relation between loaf volume and nitrogen content is set forth statistically in Table XI and Fig. 2. Linear regression accounts for most of the variation found, the correlation coefficient being $r = 0.83$. Fitting a second degree curve improved the correlation slightly but significantly to $R = 0.85$. The analyses of variance were extended to test the homogeneity of regression

TABLE XI

ANALYSIS OF VARIANCE OF LOAF VOLUME IN RELATION TO PERCENTAGE NITROGEN IN GRAIN. TEST OF SIGNIFICANCE OF REGRESSION FUNCTION

Source of variation	Degrees of freedom	Mean square
Linear regression	1	337,079**
Added effect of quadratic	1	16,615*
Residual	45	2,910

$$r_{vn} = 0.83 \qquad R_{v.nn}^2 = 0.85$$

by sub-blocks and crops, but in no case was the variance significant. It is obvious from inspection of the centroids in Fig. 2 that, as noted in the preceding paragraph, the crops fall into three groups with respect to quantitative effect on nitrogen content and corresponding loaf volume, but that there are no qualitative effects on the character of the protein. A given percentage of nitrogen in the kernels produced on the average the same loaf volume, regardless of the sod on which the wheat had been grown.

Kernel texture, expressed as percentage of hard red vitreous kernels, is an important factor in Canadian wheat grade specifications. In this experiment it was estimated by classifying a representative sample of 1000 kernels into vitreous, piebald, and starchy, first on the basis of external appearance, then by cutting the kernels with a tester. Half the percentage of piebald was added to the percentage of vitreous to get the figure for hard kernels.
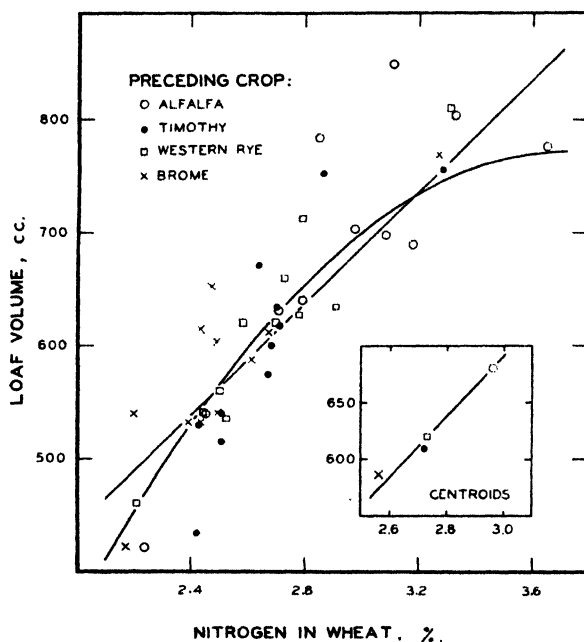

FIG. 2.   Loaf volume (bromate baking formula) in relation to nitrogen content of wheat grain.   For all data, linear relation r = 0.83; quadratic R = 0.85.

The mean values given in Table XII show the high general quality of the wheat produced on this fertile soil. The narrow range of variation in both kernel texture and protein content in a season of limited rainfall such as

TABLE XII

MEAN PERCENTAGES HARD KERNELS AND PROTEIN CONTENT (13.5% MOISTURE)

Year	May-July rainfall, in.	Hard kernels		Protein content	
		Mean	Range	Mean	Range
1930	6.24	99.2	98.8 – 99.7	16.3	15.4 – 17.1
1931	10.83	87.6	62.0 – 98.5	13.3	10.5 – 15.7
1932	7.05	97.5	91.0*– 99.9	13.1	11.4 – 15.0
1933	9.03	93.0	73.1 – 97.9	13.5	10.9 – 18.2
1934	10.01	98.8	94.9 –100.0	12.2	9.6 – 16.0

* One odd sample dropped to 78.8.

1930 restricts the likelihood of finding a high degree of correlation, and we see later (Table XIV) that the coefficient is insignificant in this case. In 1934 the diminishing fertility resulting from continuous cropping with wheat is reflected in reduced protein content, though the percentage of hard kernels is greater than would be expected from this or from the liberal May-July rainfall. On referring to the more detailed rainfall data given in the introduction, we find that the precipitation in 1934 came most abundantly in May and June, tapering off later when the texture of the kernels was developing.

Analyses of variance of percentage hard kernels are reported in Table XIII. The dominant effect of season, already evident in Table XII, is here clearly proved. Only in Sub-block II do the preceding hay crops appear to have significantly different effects on the texture of the wheat kernels, such effects being influenced differentially by seasons. Since the original data have been omitted to save space, it may be said that in Sub-block II the mean percentages of hard kernels were: after alfalfa, 98.0; after timothy, 94.5; after western rye, 96.6; after brome, 90.5. The differences were greatest in 1931, the first year after breaking, and tapered off to minimum values in 1934, the last year.

TABLE XIII

ANALYSES OF VARIANCE OF PERCENTAGE HARD KERNELS

	Variance due to	Sub-block I		Sub-block II		Sub-block III	
		D.f.	Mean square	D.f.	Mean square	D.f.	Mean square
Inter-plot comparisons	Rows	3	23.4	3	7.8	3	1.4
	Columns	3	257.8	3	36.1	3	5.4
	Crops	3	73.7	3	169.7**	3	20.7
	Error (a)	6	83.9	6	3.9	6	7.4
Intra-plot comparisons	Years	4	331.6**	3	554.8**	1	234.3**
	Crops × years	12	30.3	9	136.7**	3	13.6
	Rows × years	12	7.8	9	3.0	3	2.4
	Columns × years	12	45.8**	9	14.5**	3	4.6
	Error (b)	24	14.3	18	2.8	6	5.1

Newton, Cook, and Malloch (1) found no significant linear correlation between percentage of hard kernels and protein content over all samples in a series of six wheat varieties grown at six widely separated points in Western Canada, though within a given sample the vitreous kernels always contained more protein than the starchy kernels. The disturbing factors, they concluded, did not lie in inherent differences between varieties, but in environmental effects. In the present experiment only one variety and one station come in question, so we may look more hopefully for association of the two characteristics. In Table XIV is given an analysis of covariance of percentage of hard kernels and percentage protein content. Part A shows the linear regression of the one on the other to be highly significant over all seasons, though seasonal effects are also highly significant, as shown by the differences

in seasonal centroids and regressions. Part B gives the detailed relations in individual years. The correlation for 1930 is insignificant, as noted above, but for all the other seasons is highly significant. The average over all seasons is, $r = 0.37$; within seasons it is, $r = 0.84$.

TABLE XIV

ANALYSIS OF COVARIANCE OF HARD KERNELS AND PROTEIN CONTENT

A. Test of homogeneity of regression by seasons

Variance in kernel texture	D.f.	Mean square
Average regression all seasons	1	1152.00**
Difference in seasonal centroids	4	983.45**
Difference in seasonal regressions	4	459.17**
Residual	166	9.89

B. Relation of hard kernels and protein content in individual years

Year	Regression coefficient	Correlation coefficient
1930	+0.2% hard per % protein	.35
1931	+6.6% hard per % protein	.92**
1932	+2.3% hard per % protein	.53**
1933	+1.4% hard per % protein	.58**
1934	+0.6% hard per % protein	.62**

Average for all data, $r = 0.37$**.
Average within years, $r = 0.84$**.

Discussion

The use of the growing crop as an indicator of soil nitrification rates appeared to work at least moderately well in these experiments. Certainly the authors gained in this way a much clearer knowledge of the sequence effects of the hay crops investigated. On the other hand, the effects of timothy on succeeding crops did not turn out in this case in accordance with popular belief, nor indeed in accordance with experimental results previously obtained at the same station. The cause of this discrepancy is not immediately apparent. The results actually found are, however, in harmony with the fact that, among the grasses, timothy left in the soil the largest quantity of residue and fixed nitrogen and, because of its small yield of hay in the absence of sufficient moisture, removed the smallest quantity of nitrogen from the soil. The fact that brome was distinctly poorer than the other two grasses in its after effects raises the question as to whether such creeping rooted grasses that form a closely knit sod may depress nitrification by preventing adequate aeration of the soil. Other possible explanations will be considered in later papers of this series, and a general discussion will be more appropriate after these have appeared. The findings of other workers in the same or related fields, recorded in the literature, can be considered at the same time. One unpub-

lished observation may be mentioned here. On reading the manuscript of this paper and noting that wheat following alfalfa threw a larger proportion of straw and that the relative differences in nitrogen fixed showed up most clearly in the straw, Dr. A. G. McCalla commented that in his water culture experiments at Edmonton the wheat plants supplied with high nitrogen solutions always produced a larger proportion of straw.

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A LEAF BLIGHT OF *POPULUS TACAMAHACA* MILL. CAUSED BY AN UNDESCRIBED SPECIES OF *LINOSPORA*[1]

By G. E. Thompson[2]

Abstract

A foliage disease of *Populus tacamahaca* Mill., caused by a fungus that has been given the name *Linospora tetraspora* n. sp., is known to occur in Alberta, British Columbia, Ontario, and Quebec, Canada. The lesions on the leaves are of various sizes, dark brown in colour, with very irregular and diffused margins. The leaves may be completely invaded, discoloured, and dropped prematurely. Small, black, circular or irregular pseudoclypei develop on the upper surface of the infected leaves. No true conidial stage was found in the life history. Spermatia are produced in acervuli that develop in the cells of the upper epidermis during late summer and autumn. Isolations from ascospores and plantings of infected leaf tissues gave similar mycelial growth on potato dextrose agar. The pathogenicity of the fungus was demonstrated by the inoculation of healthy leaves with a suspension of ascospores. Symptoms typical of the disease developed in about a month. The fungus was re-isolated from the inoculated leaves.

Introduction

The author's first observations of this disease of *Populus tacamahaca* were made in the Claude River Valley, Gaspé Co., Quebec, during September 1928, and in the Temagami Forest Reserve, Ontario, during 1930–31. Later, the author received from various investigators specimens of the disease which were collected in the following localities: Sandhill, Alberta; Revelstoke, British Columbia; Iroquois Falls and Petawawa, Ontario; Duchesnay, Matapedia, and Proulx, Quebec. In a letter from Dr. J. W. Groves, it was reported as causing serious defoliation of the balsam poplar at the Petawawa Forest Experiment Station, Ontario. The disease appears to be widely distributed throughout Canada, but as far as the writer was able to determine, it has not been reported from the United States.

In the Temagami Forest Reserve, the foliage on young trees and on the lower branches of older trees was severely damaged. The leaves were discoloured and wilted, and presented the appearance of having been scorched by fire. Trees of *Populus grandidentata* Michx. and *P. tremuloides* Michx. growing in close proximity to diseased trees of *P. tacamahaca* were not affected.

Symptoms and Signs

The lesions vary considerably in size and may include the entire leaf. As infection progresses most rapidly along the main veins and spreads into the intercostal areas through the veinlets, the margins of the lesions are irregular. On the upper surface of the leaves they are dark brown. As they increase in

size, their centres become ashen or bleached in appearance. This discolouration usually extends over the entire surface when the leaf is completely invaded (Fig. 1, 1).
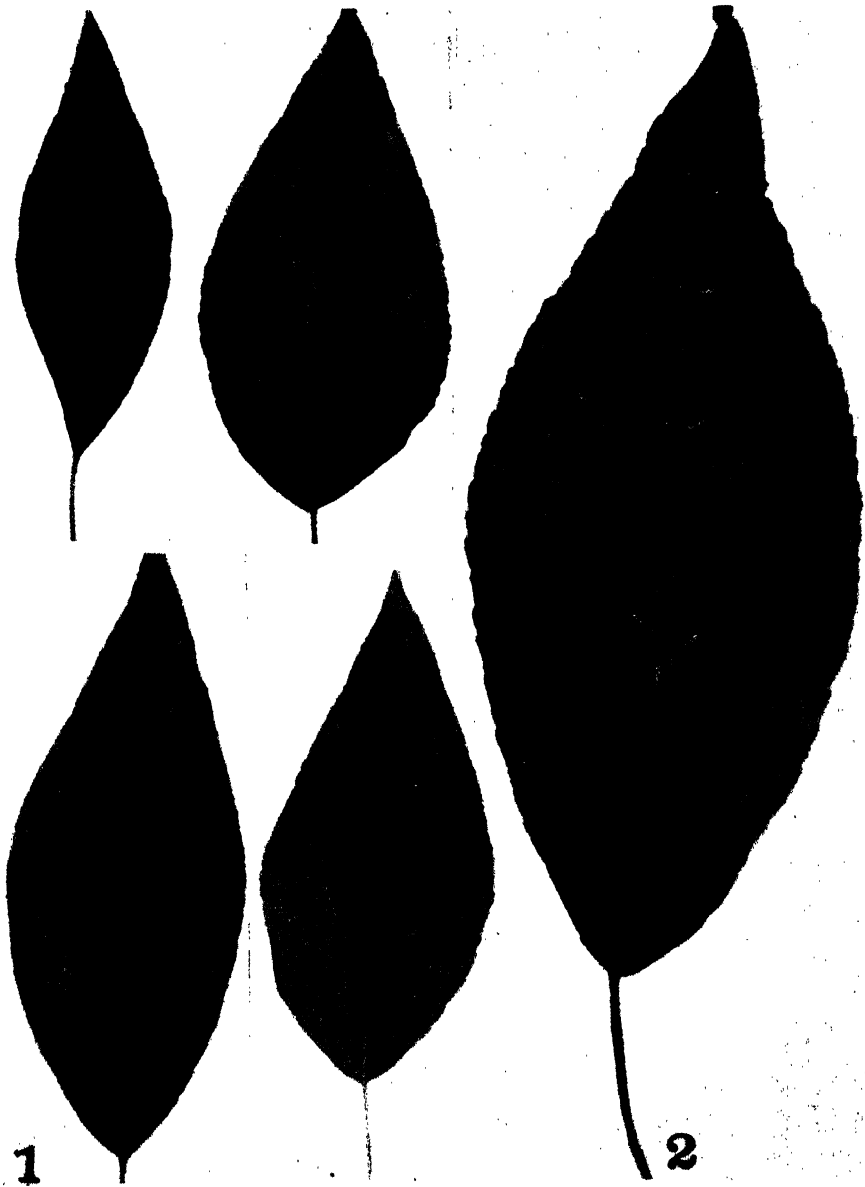

FIG. 1.   1. *Four leaves of Populus tacamahaca showing the lesions on the upper surface. Bleached areas in the centres of the lesions are visible on three of the leaves.   Natural size. 2. Lesions on a leaf showing black pseudoclypei scattered over the upper surface. ×2.   Photographed by W. R. Fisher, Department of Plant Pathology, Cornell University, Ithaca, New York.*

The lesions on the under surface of the leaves are reddish brown, and have irregular margins. The discoloured veins at the borders of the spots stand out prominently in contrast to the healthy ones. Infection usually spreads from the blades of the leaves into the petioles, which become brownish and shrivelled.

The black pseudoclypei, which are visible on the upper surface of the leaves, are of diagnostic value. They develop when the lesions become ashen coloured, and appear either singly or confluent, about 0.5 mm. in diameter, circular or irregular in outline, thickly scattered over the surface. When the leaf is completely infected, they are dispersed over the entire upper surface of the leaf (Fig. 1, 2). They are composed of dark thick-walled hyphae, which lie within the blackened epidermal cells.

Development of the Fungus

Microscopic examination shows that the mycelium in the tissues at the margins of the lesions is composed of hyaline hyphae, about 2.5 μ in diameter, which ramify between and within the cells of the leaf, most of which appear collapsed. Globules or solid masses of a brownish substance are conspicuous in the others.

CONIDIAL STAGE. That the conidial stages of *Linospora* species are not well known was pointed out by Miller and Wolf (4). They found that *Linospora Gleditsiae* Miller & Wolf had a *Gloeosporium* stage. Lindau (3) reports *Gloeosporium Tremulae* (Lib.) Passer. as the conidial stage of *Linospora populina* (Pers.) Schroeter.

Klebahn (2) found no evidence of a conidial stage in the life history of *Linospora capreae* (DC.) Fuckel.

The author was unable to find a true conidial stage associated with the fungus under investigation. However, a spermatial stage was found in the lesions during late July, August, and September, 1931. The acervuli in which the spermatia were produced were most obvious on moist leaves that had been lying on the ground. They arise within the epidermal cells of the upper surface of the leaf and are circular or irregular in outline and 125–250 μ in diameter. The hyaline spermatiophores, 9–13 $\times$ 2.5–3 μ, borne on a slight stroma, form a compact layer at the base of the acervulus. The spermatia, which appear to be budded off from the tips of the spermatiophores, are spherical, one-celled, hyaline, 2.5–3 μ in diameter (Plate I, 1). The outer walls of the epidermal cells are pushed outward and finally ruptured by the developing whitish masses of spermatia. The latter failed to germinate in sterile water or on the surface of potato dextrose agar.

PERITHECIAL STAGE: Pseudostromata, 400–750 $\times$ 200–250 μ, develop within the leaf tissues directly beneath the pseudoclypei. They consist of compact masses of hyaline pseudoparenchyma-like hyphae surrounded by two or three layers of dark-coloured hyphae. In transverse sections the pseudostromata appear as box-like compartments in which borders of dark
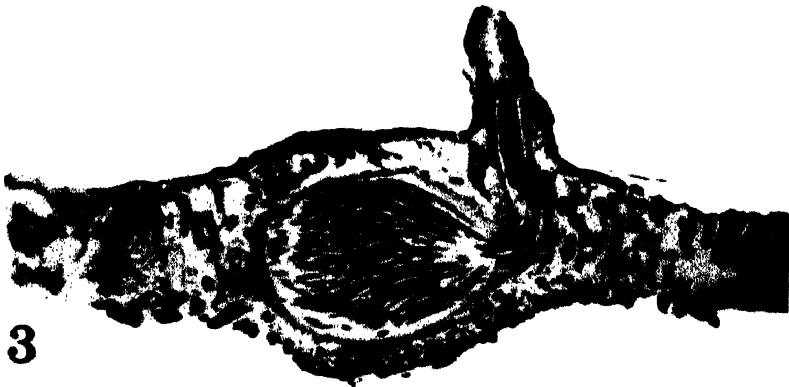
PLATE I

PLATE I. 1. *Transverse section of a leaf showing an acervulus containing a mass of spermatia and basal layer of spermatiophores.* × 270. 2. *Transverse section of a leaf showing a pseudostroma with dark margin and inner hyaline pseudoparenchyma. The remains of a few palisade cells filled with a dark-coloured deposit can be seen embedded within the pseudostroma.* ×220. 3. *Vertical section of a perithecium embedded within a pseudostroma, showing membranaceous wall, lateral beak, and long cylindrical asci.* ×125.

fungous elements surround the hyaline fungous tissue and remains of the palisade cells (Plate I, 2).

The perithecial initials consist of coils of hyaline hyphae, which originate in the centres of the pseudostromata. They were observed in stained sections of diseased leaf material, which was collected in the latter part of July. Their development concurs with that of the spermatial stage. Neither the perithecial initials nor subsequent stages of the perithecia were studied in detail.

In the Temagami region of northern Ontario, mature perithecia were found in overwintered leaves during June. They are globose or pyriform, 175–270 × 110–175 μ, usually single in the pseudostromata (Plate I, 3). The membranaceous wall of the perithecium is composed of two or three inner layers of narrow thin-walled cells and one or two layers of narrow, dark-coloured hyphal cells.

The beak of the perithecium arises laterally. It extends for a short distance into the tissues of the leaf and then curves at right angles to the leaf surface, finally projecting a short distance above it. The ostiole of the beak is lined with narrow, hyaline periphyses that extend a short distance into the perithecial cavity.

The asci are long cylindrical, straight or curved, tapering to a narrow base, rounded and thickened at the apex, 175–230 × 6.5–9 μ, four-spored (Fig. 2, A). In crushed mounts of perithecia in water, the asci separate
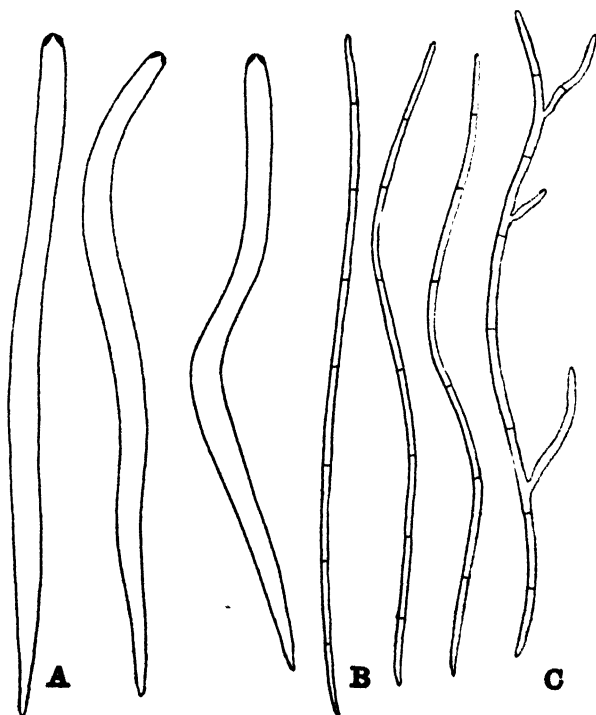
FIG. 2. *A. Asci. B. Ascospores. C. Germinating ascospore. Drawings made with the aid of a camera lucida. ×450.*

and float out freely, and their bases dissolve away as is typical of the Diaporthaceae. When perithecia are kept in a moist chamber, the asci and ascospores ooze out to form little droplets at the tips of the beaks. Both of these observations are in accordance with those of Miller and Wolf (4) for *Linospora Gleditsiae.*

The ascospores are filiform, straight or irregularly curved, six- to eight-septate, hyaline, 175–225 × 2.5–3 μ, spirally twisted within the ascus.

Paraphyses were not observed in the mature perithecia. Miller and Wolf (4) have pointed out that in *Linospora Gleditsiae*, the paraphyses disappear when the asci mature. Early stages similar to those described by Miller and Wolf were not available for study.

Taxonomic Position of the Fungus

The following characters place the fungus in the genus *Linospora* of the family Clypeosphaeriaceae: the presence of pseudoclypei; perithecia with true walls and elongated beaks; filiform, septate, hyaline ascospores, and the absence of paraphyses in the mature perithecia.

The writer was unable to find any *Linospora* described or reported as occurring on poplars in North America. Two species are known to occur in Europe: *Linospora populina* (Pers.) Schroeter on *Populus alba* L., *P. nigra* L., and *P. tremula* L.; *Linospora candida* Fuckel (1) on *P. alba* and *P. canescens* Sm.

The fungus under investigation differs from the above European species chiefly in having longer asci and ascospores, and four-spored asci. Because of these and other differences, and the fact that a *Linospora* affecting poplars has not been reported from North America, this fungus is described as a new species.

Linospora tetraspora sp. nov.

Pseudoclypeis, nigris, epiphyllis, dense sparsis, mycelio fusco in epidermide formantibus; pseudostromatis, 400–750 × 200–250 μ, inferis pseudoclypeis, formantibus hyalinis pseudoparenchymis et degeneratis palisadis cellis, marginatis atris; peritheciis, globosis vel pyriformis, 175–270 × 110–175 μ, saepissime solitariis in pseudostromatis; rostris lateris exertis atque curvulis; periphysibus in ostiolo; ascis cylindraceis, rectis vel curvulis, contractis imo, apice incrassito, 175–230 × 6.5–9 μ, 4-sporis; ascosporis filiformibus, rectis vel irregulariter curvatis, 6–8 septatis, hyalinis, 175–225 × 2.5–3 μ, spiraliter contortus in ascis.
Hab. in foliis dejectis vere *Populi tacamahacae* Mill.
Status spermaticus: Acervulis, epiphyllis, intraepidermide, orbicularibus vel irregularibus, 125–250 μ diam.; hyphis fertilibus, hyalinis, 9–13 × 2.5–3 μ, spermatiis sphaericis, continuis, hyalinis, 2.5–3 μ diam.
Hab. in foliis vivis et in foliis dejectis aestate sera et autumne *Populi tacamahacae* Mill.

TYPE LOCALITY: Sandy Inlet, Lake Temagami, Temagami Forest Reserve, Ontario.

DISTRIBUTION: British Columbia, Alberta, Ontario, and Quebec.

TYPE SPECIMENS: Author's collection number 556, part of which has been deposited in the following herbaria: Department of Botany, University of Toronto, Toronto, Ontario; Department of Plant Pathology, Cornell University, Ithaca, New York; and Farlow Herbarium, Harvard University, Cambridge, Massachusetts.

Cultures

Pieces of overwintered leaves containing mature perithecia were fastened to the covers of Petri dishes in such a way that ascospores discharged from perithecia fell on the surface of the medium below. Germination of the ascospores took place within 24 hr. Germ tubes were formed at either the ends or the sides of the ascospores (Fig. 2, C).

Numerous transfers of single ascospores and masses of ascospores were made on test tube slants of potato dextrose agar. The single ascospores failed to grow, and only a few of the mass ascospore plantings developed into colonies. The mycelia grew very slowly, forming whitish colonies which later became brownish in colour. The hyphae tended to build up in the centres of the colonies. When the cultures were examined microscopically, they were found to consist of hardened masses of short, thick-walled brownish hyphae. Neither spores nor fruiting structures of the fungus were found in the cultures.

A few successful isolations of the fungus were obtained from tissue plantings taken from the margins of lesions on diseased leaves. The colonies were similar to those derived from ascospores.

Inoculations

On June 23, 1931, mature perithecia were dissected out of overwintered leaves and crushed out in sterile water. The suspension of asci and ascospores thus obtained was transferred to an atomizer that had been rinsed out previously with 95% alcohol and sterile water. A small tree of *Populus tacamahaca* growing in a location free from the disease was selected for inoculation. The terminal leaves on each of three branches were enclosed in celluloid cylinders. The end of the cylinder nearest the trunk was plugged with moist sphagnum. The suspension of asci and ascospores was atomized on the leaves through the open end of the cylinder, which was then plugged with moist sphagnum. The cylinders were left on the branches for three days. During this period the sphagnum was kept moistened.

The artificially inoculated leaves developed symptoms typical of those found on naturally inoculated leaves by July 23, 1931. A few weeks later pseudoclypei and pseudostromata developed in the leaf tissues. The surrounding foliage on the tree remained free from infection. On July 25, 1931, the fungus was re-isolated from the diseased tissues of the inoculated leaves. The colonies that developed from these isolations were similar to those made from ascospores. The results of these experiments demonstrate the pathogenicity of the fungus.

Acknowledgments

The investigations reported in this paper were conducted at the Department of Botany, University of Toronto, under the direction of Professor H. S. Jackson, to whom the author is grateful for continued interest and valuable suggestions. He extends his thanks to Dr. J. H. Miller, Department of Plant Pathology, University of Georgia, Athens, Georgia, for his critical reading of the manuscript.

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VARIETAL DIFFERENCES IN BARLEYS AND MALTS

VI. AUTOLYTIC PROTEOLYTIC ACTIVITY OF MALT AND ITS CORRELATIONS WITH WORT NITROGEN AND BARLEY NITROGEN FRACTIONS[1]

By C. ALAN AYRE[2] AND J. ANSEL ANDERSON[3]

Abstract

The proteolytic activity of 144 samples of malt, representing 12 varieties grown at 12 experimental stations in Canada, was determined by an autolytic method. Certain varieties differed widely in average activity (Olli, 291; O.A.C. 21, 235; and Wisconsin 38, 150 units), those of poor malting quality tending to give low values. The spread between station means was also large (Beaverlodge, 284; and Nappan, 149 units).

The correlations between proteolytic activity, barley nitrogen fractions, total barley nitrogen, and wort nitrogen (data given in an earlier paper), were also studied. *Intra-varietal* partial correlations independent of total nitrogen, between proteolytic activity and nitrogen fractions, were all insignificant. Corresponding *inter-varietal* partial correlations were insignificant for insoluble and alcohol-soluble nitrogen, but highly significant for salt-soluble barley nitrogen and wort nitrogen. A close *inter-varietal* relation was found between proteolytic activity and salt-soluble barley nitrogen, and it was impossible to demonstrate that these two properties influenced wort nitrogen independently.

Varietal differences in the percentage of barley nitrogen appearing in the wort extracted from malts, or differences in some fraction of this wort nitrogen, have been demonstrated by several investigators (3, 4, 12, 13, 15, and others), and it has seemed reasonable to suppose that these differences reflected differences in the amounts of proteolytic enzymes elaborated or liberated during the malting process. In the preceding paper of this series (2), however, it was shown that a fairly high inter-varietal relation ($r = 0.88$) existed between wort nitrogen and the salt-soluble nitrogen of the barley from which the malt is made. It thus appeared that wort nitrogen might be a function of both salt-soluble barley nitrogen and proteolytic activity, and might therefore be a rather poor measure of the latter. Alternatively, it also seemed possible that both salt-soluble barley nitrogen and wort nitrogen might be functions of proteolytic activity, and that the correlation between the two nitrogen fractions might be merely an expression of these relations.

The work reported in this paper, a study of the autolytic proteolytic activity of 12 barley varieties, besides demonstrating that large varietal differences exist, also provided data bearing on the questions discussed above. Statistical analyses showed that a close inter-varietal relation exists between salt-soluble barley nitrogen and proteolytic activity of malt, but it was not possible to demonstrate that these two properties influenced wort nitrogen independently.

Materials

The malts used in this study are those used in previous studies in this series. They represent 12 varieties of barley grown at 12 widely separated experimental stations in Canada (listed in Table I). The barley varieties and the methods used in growing the samples were described in detail in Part I (1) of this series and the malting methods and commonly measured characteristics of the malts were reported in Part IV (12).

Method

A considerable number of methods for evaluating the proteolytic activity of malts have been developed. In the majority of these an aqueous extract of the malt is made, and its activity is measured by the change in viscosity of a gelatin substrate (6, 9, 10), or by its hydrolytic action on an edestin substrate (7, 9). Laufer (9) reports that no agreement exists between the results of representative gelatin and edestin methods. Idoux (5) used ground barley, in which enzymes were said to have been destroyed by drying at 115° C. for 4 hr., as a substrate. Kolbach and Simon (7) used an autolytic method in which the malt protein acts as substrate. They attribute the greater portion of proteolytic activity to insoluble enzymes and have shown that results obtained from an autolytic digestion differ widely from those obtained by allowing an aqueous extract of the malt to act on edestin. They prefer the autolytic method. Lüers (11) also recommends the use of the natural malt protein substrate.

The method selected for the present investigation involved measuring the amount of non-protein nitrogen produced during the second and third hours of digestion in a malt mash buffered to pH 4.6 and maintained at 45° C.

The method is by no means free from objections. Differences in the protein distribution among samples of the same variety grown at different stations make the interpretation of station differences in autolytic proteolytic activity difficult, if not impossible. On the other hand, since inter-varietal differences in protein distribution are reasonably small, comparisons of auto-lytic values for samples of different varieties grown at the same station can probably be considered fairly reliable.

Typical curves showing the relations between non-protein nitrogen produced and time of digestion, for samples of two varieties grown at the same station, are shown in Fig. 1. These show that the method is not particularly precise, that the rate of proteolysis is only approximately constant over the period selected for measurement (1 to 3 hr.), and that the rate begins to decrease with longer periods of digestion.
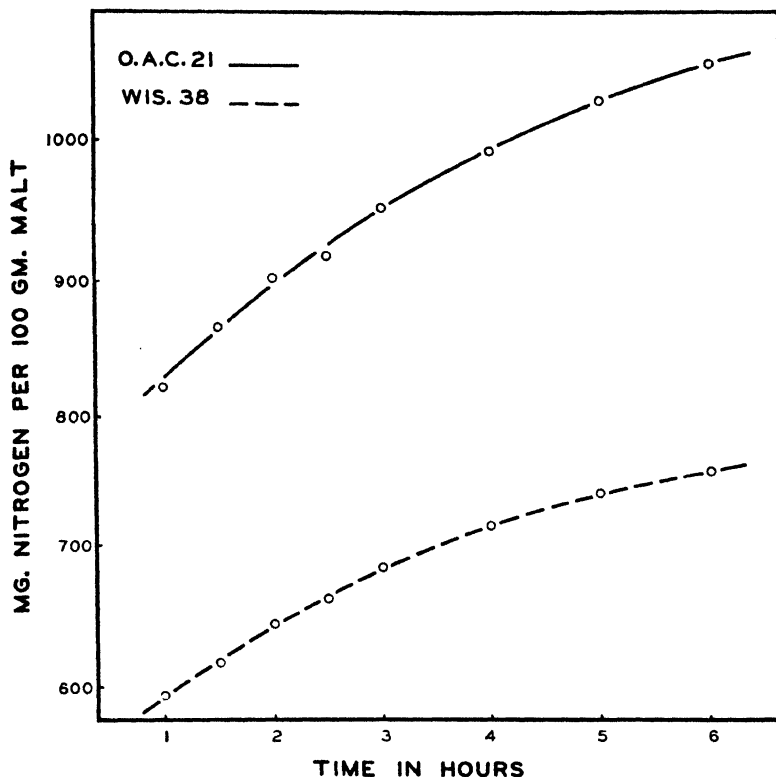
Fig. 1. *Non-protein nitrogen produced by autolytic digestion of malts for successive time intervals.*

Details of Method. A 25-gm. sample of malt was finely ground in a Seck mill. Two 10-gm. aliquots were weighed into 100-ml. glass centrifuge tubes, and 50 ml. of a sodium acetate-acetic acid buffer solution, pH 4.6, were added to each tube with rapid stirring. The tubes were placed in a water bath at 45° C., and the mashes were stirred at 5-min. intervals.

The proteolysis in one aliquot was stopped after 1 hr. by adding 10 ml. of 30% trichloracetic acid solution. After stirring and centrifuging, the supernatent liquid was filtered and the nitrogen (*i.e.*, non-protein nitrogen) in 20 ml. of the filtrate was determined by a Kjeldahl method. This determination replaces the blank determination. It was made after 1 hr. of digestion, rather than earlier, in order to allow the original soluble constituents of the malt sufficient time to dissolve completely.

The proteolysis in the second aliquot was stopped after 3 hr., and the non-protein nitrogen was determined as previously described.

Proteolytic activity is calculated from the difference between the amounts of non-protein nitrogen in the 1- and 3-hr. digestions.

Replication. In order to provide a check on the precision of the determination during the investigation, duplicate determinations were made on

one-third of the samples.   These were selected at random, after imposing the limitation that four samples of each of the 12 varieties and four samples from each of the 12 stations should be chosen.   The standard deviation of the mean of duplicate determinations, calculated from 48 pairs of values, was ± 13.2 for a mean value of 220 mg. of non-protein nitrogen, per 2 hr., per 100 gm. of malt.   Precision did not prove to be a limiting factor in the comparisons of either varietal or station means.

Results and Discussion

Varietal and Station Differences

The results of the investigation are summarized in Table I as varietal means, over all stations, and as station means, over all varieties.   Owing to the differential effect of environment on varieties, these did not fall in the same order with respect to proteolytic activity at all stations.   For the same reason the stations did not fall in the same order within each variety. An analysis of variance was necessary to determine whether the differences between varietal and station means could be considered significant.   The results of the analysis are recorded in Table II and show that both varietal and station differences are highly significant.   To facilitate comparisons the necessary differences between means for a 5% level of significance, i.e., for odds of 19 to 1 that a real difference is operating to spread the means, are given in the last line of Table I.

TABLE I

PROTEOLYTIC ACTIVITY OF MALTS: MEANS FOR EACH VARIETY AND EACH STATION

Class	Variety	Proteo-lytic activity*	Station	Proteo-lytic activity*
Six-rowed, rough-awned	O.A.C. 21	235	Nappan	149
	Mensury, Ott. 60	230	Fredericton	192
	Olli	291	Ste. Anne de Bellevue	199
	Peatland	220	Ste. Anne de la Pocatiere	218
	Pontiac	214	Lethbridge	195
Six-rowed, smooth-awned	Nobarb	100	Winnipeg	225
	Regal	196	Brandon	213
	Velvet	210	Guelph	219
	Wisconsin 38	150	Ottawa	210
Two-rowed, rough-awned	Charlottetown 80	264	Lacombe	244
	Hannchen	225	Beaverlodge	284
	Victory	196	Gilbert Plains	242
Necessary difference, 5% level		29		29

* Proteolytic activity is reported as milligrams of non-protein nitrogen produced by 100 gm. of malt during the second and third hours of an autolytic digestion.

Comparison of the actual differences between varietal means with the necessary difference leaves no room for doubt that proteolytic activity, as measured in the present investigation, is a varietal characteristic.   These

results are thus in line with those of Wahl (14) and Koch *et al.* (6) who have previously made small studies of varietal differences in proteolytic activity using other methods of measurement.

TABLE II

ANALYSIS OF VARIANCE FOR PROTEOLYTIC ACTIVITY

Variance due to	Degrees of freedom	Mean square
Varieties	11	18,415.8**
Stations	11	13,103.0**
Remainder	121	1,306.9

NOTE: *In this and later tables,* ** *denotes that the 1%
level, and* * *that the 5% level of significance is attained.*

There is no evidence that different classes of barley are characterized by differences in proteolytic activity. The rough-awned, six-rowed variety, Olli, gives the highest values and is followed by the two-rowed variety, Charlotte-town 80. Eight of the other varieties form an intermediate group covering a range of 40 units. Two of the smooth-awned, six-rowed varieties, Nobarb and Wisconsin 38, give very low values.

In Canada it is generally agreed that among the six-rowed, rough-awned varieties, O.A.C. 21, Mensury and Olli are best for domestic malting purposes, and that among the smooth-awned group, Velvet is much less unsatisfactory than the others. The data in Table I thus indicate that fairly high proteolytic activity is an attribute of varieties of good malting quality.

A study of the station means also suggests that proteolytic activity is affected by environment. The stations are listed in order of increasing nitrogen content of their samples (*cf.* (1, Table I)). Inspection will thus show an apparent increase in proteolytic activity with increasing total nitrogen. It should be borne in mind, however, that increasing amounts of nitrogen substrate together with regular changes in the distribution of the nitrogen among various protein fractions (*cf.* (1, Table II)), may affect the apparent proteolytic activity. It would thus be unwise to attempt to draw definite conclusions about the effect of environment on proteolytic activity from the data presented in Table I.

The authors have considered the possibilities of correcting for the effect of differences in the nitrogen contents of samples from different stations, either by reporting proteolytic activity in terms of the percentage of nitrogen hydrolyzed, or by making adjustments by means of the analysis of variance and covariance method (*cf.* (1, p. 388)). It appears, however, that to make corrections by either method, certain unjustifiable assumptions must be made. In these circumstances it seems preferable to let further research elucidate the issues involved.

Relations between Proteolytic Activity and Nitrogen Fractions

Correlation coefficients showing the relations between proteolytic activity on the one hand, and total barley nitrogen, barley nitrogen fractions, and wort nitrogen, on the other, are given in Table III. The independent variables are listed in the first column, and the relation between proteolytic activity and each of these is represented by a row of four correlation coefficients, namely, the simple and partial correlation coefficients for varieties and stations.

TABLE III

RELATIONS AMONG PROTEOLYTIC ACTIVITY (p) NITROGEN FRACTIONS (x) AND TOTAL NITROGEN (n)

x = independent variables listed below	Correlation coefficients			
	Varieties		Stations	
	Simple r_{px}	Partial $r_{px.n}$	Simple r_{px}	Partial $r_{px.n}$
Total nitrogen	.070	---	.854**	---
Insoluble protein nitrogen	−.474	−.539	.792**	.006
Alcohol-soluble protein nitrogen	−.098	−.265	.888**	.297
Total salt-soluble nitrogen	.871**	.881**	.578*	−.545
Salt-soluble protein nitrogen	.870**	.871**	.545	−.235
Non-protein nitrogen	.741**	.780**	.683*	−.064
Wort nitrogen	.874**	.877**	.607*	−.136
Residual degrees of freedom	10	9	10	9

Comparison of the data given in the first two columns of Table III shows that inter-varietal differences in total nitrogen content have very little effect on the inter-varietal relations between proteolytic activity and nitrogen fractions. The correlation coefficients have essentially the same values irrespective of whether an adjustment is or is not made for varietal differences in total nitrogen content. The statistics show that no inter-varietal correlation exists between proteolytic activity and total nitrogen, insoluble protein nitrogen or alcohol-soluble nitrogen. On the other hand, it is shown that proteolytic activity is directly correlated with the more soluble barley nitrogen fractions and with wort nitrogen: varieties that tend to be high in proteolytic activity also tend to be high in content of salt-soluble barley nitrogen, and tend to produce· malts yielding worts of higher nitrogen content.

The correlation coefficients for stations present a different picture. The fact that the simple correlation coefficients are significant, whereas the partial correlation coefficients are not, shows that the former merely reflect the correlations between proteolytic activity and total nitrogen, and between the independent variables and total nitrogen (*cf.* (1, Table VI)). When the complicating effect of station differences in total nitrogen is removed by calculating partial coefficients, it becomes apparent that the experimental data fail to demonstrate that any intra-varietal relation exists between proteolytic activity and the various nitrogen fractions. The data show only

that within varieties, environmental conditions that tend to increase total nitrogen content also tend to increase proteolytic activity.

Relations between Wort Nitrogen, Proteolytic Activity and Total Salt-soluble Barley Nitrogen

In Part V of this series (*cf.* (2, Table I)), it was shown that a highly significant intervarietal correlation ($r = 0.887$) exists between wort nitrogen and total salt-soluble barley nitrogen. Since inter-varietal correlations of the same magnitude exist between both these nitrogen fractions and proteolytic activity (see Table III), further investigation of the inter-relations among these three properties seemed desirable. The correlation coefficients given in Table IV help clarify these relations. The simple correlation between

TABLE IV

RELATIONS BETWEEN WORT NITROGEN, PROTEOLYTIC ACTIVITY, AND TOTAL SALT-SOLUBLE BARLEY NITROGEN

Correlation between	Correlation coefficient
Wort nitrogen and salt-soluble barley nitrogen	
Simple	.887**
Partial, independent of proteolytic activity	.528
Wort nitrogen and proteolytic activity	
Simple	.874**
Partial, independent of salt-soluble barley nitrogen	.448

wort nitrogen and salt-soluble nitrogen is highly significant, but the partial correlation, independent of proteolytic activity, is insignificant. The relation between wort nitrogen and proteolytic activity is very similar: the simple correlation is significant, but the partial correlation independent of salt-soluble nitrogen is insignificant. These relations appear to show that salt-soluble nitrogen and proteolytic activity are directly related; because, when the covariance between salt-soluble nitrogen and the other two factors, *i.e.*, proteolytic activity and wort nitrogen, is removed, most of the covariance between the two latter factors is removed also. It should also be noted that this relation makes it impossible to obtain a significantly higher correlation between wort nitrogen and salt-soluble nitrogen by introducing proteolytic activity as a second independent variable. The multiple correlation coefficient proved to be 0.891, which is not significantly higher than the simple correlation, 0.887.

In these circumstances it is impossible to differentiate between the parts played by the proteases and the salt-soluble nitrogen fraction of barley in producing wort nitrogen. All that can be said is that varieties that tend to contain larger amounts of salt-soluble nitrogen also tend to produce malts of higher proteolytic activity which yield worts of higher nitrogen content.

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PHYSIOLOGICAL ACTIVITY OF A SERIES OF NAPHTHYL ACIDS[1]

By N. H. Grace[2]

Abstract

An homologous series of ω-naphthyl, aliphatic acids from the acetic to the hexoic has been presented to the author by Dr. R. H. Manske, and the physiological activity of these has been determined by the rooting response of plant cuttings treated with solutions of each. Statistically significant positive effects have been noted on the number of cuttings that rooted, the number and length of roots per rooted cutting, and the mean root length. The results with several plant species indicate that activity exists up to and including naphthyl hexoic acid, the highest member of the series tested. A noteworthy feature of the results is the activity of the acids with an even number of carbon atoms in the side chain; those with an odd number have activity of a lower order.

An homologous series of naphthyl acids has been prepared and reported by Dr. R. H. F. Manske (3). It has been observed that 1-naphthylacetic acid possesses properties similar to those of indolyl-3-acetic acid in affecting certain plant responses, and that a mixture of 1- and 2-γ-naphthylbutric acid also has a measure of activity (2, 5). It is therefore of both theoretical and practical interest to determine the activity of other members of the series. Accordingly, the physiological activity of the acids from 1-naphthylacetic to ϵ-(1-naphthyl)-hexoic has been determined by the rooting response of treated cuttings. In addition, the series of treatments included naphthylene-1, 5-diacetic acid, indolyl-3-acetic acid, and a control, making eight treatments.

Experimental

The activity of the various acids was determined by the rooting responses of both summer collections of greenwood and herbaceous material and winter collections of dormant current year's growth.* Cuttings were treated by immersing the basal end in solution for a period of 22 hr. for summer, and 24 hr. for dormant, material. Controls were treated in a 100 p.p.m. solution of potassium acid phosphate; solutions of the acids were prepared in phosphate of this concentration, as solubility in water is extremely low for some of the members of the series.

In the earlier experiments the acids were used at five concentrations, 100, 50, 25, 10, and 5 p.p.m. of solution. There were three replicates, with seven cuttings in each group, but in one experiment in which the material was limited, five cuttings were used for each of the eight treatments. The total number of cuttings was 840. In some of the earlier experiments individual treatments were carried out in separate beakers, enabling determination of

the change in weight accompanying treatment. Subsequently, all the replicates of one treatment and concentration were treated together. In a later experiment, with dormant cuttings of *Lonicera tartarica*, there were ten cuttings to the group with only three concentrations, 100, 50, and 10 p.p.m. In this experiment there were five replicates and 1200 cuttings. The amount of solution used in treatment varied from 50 cc. for groups of seven small cuttings to 150 cc. when five replicates of ten large cuttings were treated in the same beaker, but was constant throughout any given experiment.

The design of the experiment provided for analyses of variance of the observations. Each replicate, or complete block, contained five incomplete blocks, each comprising the eight treatments at one concentration in random order. Treatments were further randomized within incomplete blocks. All the summer cuttings were planted in brown sand in cotton-covered propagation frames in a garden. Dormant winter cuttings were placed in brown sand in frames equipped with bottom heating electrical cables and situated in a greenhouse. The room temperature approximated 65° F., while the sand temperature was maintained at 72° F. When the cuttings were removed, record was made of the number rooted, the number and length of roots per rooted cutting and, in some experiments, the mean root length.

Results

Each experiment was taken from the propagation frame when preliminary observation indicated that substantial rooting had occurred. The results described in the following paragraphs are arranged under the headings of the different plants used.

Viburnum Opulus L.

Cuttings of this plant were treated August 5 and removed from the propagation frame September 10, 1938. Measurements were made immediately on the rooted cuttings.

The data in Table I are for the change in weight of groups of seven cuttings that had received a 22-hr. solution treatment, for the number of cuttings rooted, and for the mean number of roots per rooted cutting. Statistically significant effects on the weight of cuttings occurred following treatment with each of the six naphthyl acids and with indolylacetic acid. There are significant differences within concentrations of each of the naphthyl acids. The butyric and valeric acids cause loss in weight, while naphthyl hexoic shows both marked gain and loss in weight over the concentration range tested. This fact is of interest, as the tendency to form colloidal solutions becomes marked with naphthyl butyric acid and increases with lengthening side chain. Increased rooting follows weight increase with indolylacetic acid at 10 p.p.m. Weight increase of cuttings on treatment with the naphthyl acids either reduces or does not affect the number of cuttings rooted. The hexoic acid at 25 p.p.m. is an exception.

There are significant effects on rooting from each of the chemical treatments at one or more of the concentrations used, with the valeric acid the sole

TABLE I

RESPONSES OF *Viburnum Opulus* CUTTINGS TREATED IN SOLUTIONS OF CHEMICALS

Data are means of three groups of seven cuttings

Concentration, p.p.m.	Control	Indolyl-acetic acid	Acids of naphthyl series						Necessary difference, 5% level
			Acetic	Propionic	Butyric	Valeric	Hexoic	Diacetic	
Change in weight on treatment, gm.									
100	0.51	0.47	1.50*	1.10*	0.13	−1.13*	0.47	0.33	0.69 between chemicals at one concentration, 0.53 between control and chemicals, and 0.31 between means of chemicals
50	0.51	0.47	0.60	0.87	−0.57*	−0.63*	1.47*	1.03	
25	0.51	1.00	0.10	0.87	−0.40*	−0.23*	1.27*	1.43*	
10	0.51	1.10*	0.63	0.63	−0.47*	−0.03*	0.70	0.80	
5	0.51	0.73	0.67	0.37	−0.17*	−0.30	−0.43*	0.60	
Mean	0.51	0.80	0.70	0.77	−0.29*	−0.35*	0.69	0.84*	
Number of cuttings rooted†									
100	2.6	2.7	1.0*	4.0	2.3	2.7	5.0*	4.7*	
50	2.6	1.7	1.7	3.0	5.3*	4.0	2.7	2.3	
25	2.6	3.0	1.7	5.3*	3.3	2.7	5.0*	3.3	
10	2.6	5.0*	5.3*	4.3	4.3	3.7	5.0*	5.3*	
5	2.6	4.3	4.7*	3.7	4.7*	4.7	4.7*	1.0*	
Mean	2.6	3.3	2.9	4.1*	4.0*	3.5	4.5*	3.3	
Number of roots per rooted cutting									
Mean	5.1	9.4*	8.8*	5.2	7.1	4.8	6.1	5.8	2.14

*Values significantly different from the control.

†Data transformed to $\sqrt{x + \frac{1}{2}}$ basis (1); necessary differences cannot be given on the untransformed means given above.

exception. The mean number of rooted cuttings over all concentrations shows naphthyl propionic, butyric, and hexoic acids to have an effect. The relative effects of the chemicals on rooting is observed most clearly from the body of the table, since there is a significant interaction between chemical treatment and concentration. It may be noted that the mean over all concentrations is affected by damage from overdosage, as shown by the 100 p.p.m. treatment with naphthyl acetic acid. Perhaps the most interesting feature of the data is the concentration range over which rooting is stimulated. Naphthyl acetic is damaging at the 100 p.p.m. level and shows a stimulating effect at the 10 and 5 p.p.m. concentrations; naphthyl hexoic is stimulating over the range from 100 p.p.m. down to 5 p.p.m. The results suggest that either the butyric or hexoic acids could be used over a wider range than the acetic, avoiding some of the danger from overdosage associated with the use of solutions of naphthyl acetic acid on cuttings. It must be pointed out that the chemicals are compared on the basis of absolute weight; but the use of equivalent weights would not materially alter the general conclusion.

Analysis of variance of the observations on the number of roots per rooted cutting indicated that the only significant effect was that of chemicals over all concentrations, this being significant to the 1% level. Significant increase in the number of roots follows treatment with both indolyl and naphthyl acetic acids. The number obtained with the butyric acid is not significantly below that with naphthyl acetic and is greater than that with valeric acid. It is apparent that the acids with an even number of carbon atoms in the side chain produce more roots on those cuttings that root than do the acids with an odd number.

Analysis of the observations on the root length per rooted cutting indicated that the data were not significant.

Coleus Blumei Benth.

Cuttings of this plant were treated on August 24 and removed from the propagation frame for measurement on September 23, 1938. The data on the number of rooted cuttings were not analyzed statistically, as this herbaceous plant roots readily and only five cuttings were used to a group. In consequence, the data presented deal with the responses of those cuttings that actually rooted.

In Table II are given data for the number and lengths of roots per rooted *Coleus Blumei* cutting. Considering means over all concentrations it is apparent that naphthyl acetic, propionic, and hexoic acids have significantly increased the number of roots. Each of the acids, excepting the hexoic and diacetic, increases the number of roots at one or more concentrations, the most pronounced effect being that of naphthyl acetic acid, which is effective at all concentrations but 5 p.p.m. and has a much greater effect than indolylacetic acid. There is an apparent falling-off in effect with those acids having four and five carbon atoms in the side chain, activity becoming more pronounced again at the member with six in the chain.

TABLE II

RESPONSES OF *Coleus Blumei* CUTTINGS TREATED IN SOLUTIONS OF CHEMICALS

Data are means of three groups of five cuttings

Concentration, p.p.m.	Control	Indolyl-acetic acid	Acids of naphthyl series						Necessary difference, 5% level,
			Acetic	Propionic	Butyric	Valeric	Hexoic	Diacetic	
Number of roots per rooted cutting									
100	5.9	12.0*	23.9*	9.0	8.1	6.5	11.4*	7.3	3.6 between mean of control and chemicals, 2.1 between means
50	5.9	9.6*	15.7*	10.2*	5.9	7.5	8.4	5.7	
25	5.9	4.7	10.9*	7.0	4.8	4.2	7.5	4.3	
10	5.9	4.7	12.4*	10.0*	4.2	9.5*	3.7	4.9	
5	5.9	6.2	8.6	9.4	6.7	7.9	8.9	4.9	
Mean	5.9	7.5	14.9*	9.1*	6.0	7.1	8.0*	5.4	
Root length per rooted cutting, mm.									
Mean	154	153	263*	279*	150	207	234	168	83
Mean root length, mm.									
Mean	24	21	19*	29*	24	28	28	29*	4.7

Values significantly different from the control.

The only data that are significant for the root length per rooted cutting are the means over all concentrations of the chemicals. These means are significant to the 1% level. Significance may be attributed to the increase in total length of roots resulting from treatment with naphthyl acetic and propionic acids. The effect appears to fall with naphthyl butyric acid, rising with the hexoic, which, while not significantly above the control, gives a greater length of root than the butyric. It is of interest to note that indolylacetic acid failed to show significant effects.

The only significant feature of the data for mean root length is the mean over all concentrations of the chemicals. These are significant to the 0.1% level. It is apparent that treatment with naphthyl acetic acid reduces the length of the individual root; the reduction effected by indolylacetic acid fails to reach significance. The remaining naphthyl acids all increase the mean root length. The increase attains significance with the propionic and diacetic acids. This increase of mean root length is interesting, as solution treatment with growth-promoting substances usually shortens the length of the individual root.

Lonicera tartarica L.

Dormant stem cuttings of *Lonicera tartarica* were treated with solutions of the chemicals on October 18, 1938, and removed for observation 48 days later. The cuttings were approximately 10 in. in length, and were collected before the canes had been subjected to any appreciable frost.

Data are given in Table III for the number of cuttings rooted, the number of roots per rooted cutting, the root length per rooted cutting, and the mean root length. Most of the data attain the 0.1% level of significance. The effect of concentrations on the mean root length is the only one in which merely the 5% level is reached.

All the chemical treatments significantly increase the number of cuttings rooted. Concentrations also are significant, the 100 and 50 p.p.m. levels both giving better rooting than the 10 p.p.m., but not differing between themselves. Since there is no significant interaction between chemicals and concentrations, mean values for rooting over the three concentrations are given. Naphthyl acetic and butyric acids are significantly more effective than either the propionic, valeric, or diacetic acids; the hexoic, however, is not significantly less effective than the acetic or butyric. Naphthyl butyric acid causes significantly better rooting than all other treatments excepting the acetic and hexoic members of the series. Chemicals and concentrations are both very highly significant in their effect on the number of roots per rooted cutting, and the interaction between them is also very highly significant, passing the 0.1% level. The 10 p.p.m. concentration is significantly less effective than the other two levels, which do not differ between themselves. Indolylacetic acid and those naphthyl acids with an even number of carbon atoms in the side chain are significantly more active than the others. Naphthyl valeric acid shows some activity, but of a lower order. The effect of indolyl-acetic acid, and naphthyl acetic, butyric, and hexoic acids increases in a

TABLE III

RESPONSES OF Lonicera tartarica CUTTINGS TREATED IN SOLUTIONS OF CHEMICALS

Data are means of five groups of ten cuttings

Concentration, p.p.m.	Control	Indolyl-acetic acid	Acids of naphthyl series						Mean value for concentration	Necessary difference, 5% level
			Acetic	Propionic	Butyric	Valeric	Hexoic	Diacetic		
Number of cuttings rooted, transformed data (1)										
Mean	2.50	2.94*	3.10*	2.86*	3.14*	2.88*	2.95*	2.73*	—	0.20
Number of cuttings rooted of 10 planted										
Mean	5.9	8.2	9.1	7.8	9.4	7.9	8.3	7.0	—	
Number of roots per rooted cutting										
100	2.0	8.3*	9.7*	3.1	9.6*	4.3*	5.9*	2.8	5.69	Interaction 1.60, means 0.92, concentrations 0.59
50	2.1	6.1*	9.4*	3.0	8.6*	3.0	6.4*	2.5	5.12	
10	2.8	2.8	5.5*	3.1	5.5*	2.8	3.5	2.4	3.55	
Mean	2.3	5.8*	8.2*	3.0	7.9*	3.4*	5.2*	2.6	—	
Root length per rooted cutting, mm.										
100	128	421*	430*	199	437*	251*	373*	170	301	Interaction 108, means 62, concentrations 22
50	144	353*	443*	191	472*	167	444*	181	299	
10	192	173	333*	214	372*	164	232	166	231	
Mean	155	316*	402*	201	427*	194	350*	172	—	
Mean root length, mm.										
Mean	70	57*	51*	68	56*	60*	66	67	—	9.3

*Values significantly different from the control.

pronounced manner with concentration. The other acids fail to show this response.

Chemicals, concentrations, and the interaction between them show highly significant effects on the root length per rooted cutting. The 10 p.p.m. concentration is again significantly below that for the two higher concentrations, which do not differ between themselves. Indolylacetic acid and the naphthyl acids with an even number of carbon atoms in the side chain are significantly more effective than all the other treatments. Naphthyl butyric acid is the most active chemical, but is not significantly better than naphthyl acetic. Total root length increases with rising concentration of indolylacetic and naphthyl acetic, butyric, and hexoic acids. However, this interaction between chemical and concentration is not shown by the other members of the series.

The mean root length is reduced by all chemicals excepting naphthyl propionic, diacetic, and hexoic acids. This time the average of the highest concentration, 100 p.p.m., differs significantly from that of the other two, which do not differ between themselves. There is no interaction between chemicals and concentrations. It is apparent that solution treatment with physiologically active chemicals tends to reduce the length of the individual root. It is interesting to note that the hexoic acid, highly active in other respects, fails to show this effect. This may be due to marked change in transport as the length of the side chain increases. Since damage is frequently indicated by the production of masses of short roots by the cutting, it is possible that naphthyl hexoic acid might be used with somewhat less danger of damage from overdosage.

There were significant differences between replicates for both the root length per rooted cutting and the mean root length. Block differences were not significant for the number of cuttings rooted or the number of roots per rooted cutting. It frequently has been observed that root length measurements are subject to fairly marked block differences.

Discussion

The six acids tested all have a measure of activity similar to that of indolylacetic acid, a recognized plant-growth-stimulating chemical. The most precise results were obtained with dormant stem cuttings of *Lonicera tartarica*. This fact is due, in part, to the increased replications used in this particular experiment. It is impossible to make any close comparison of the results obtained with the three different plants; however, significant differences in activity were brought out by each.

It may be pointed out that physiological activity of the chemicals has been demonstrated by two distinct types of observation. The first deals with the initiation of roots, a fact of prime importance in the practical application of chemicals for this purpose. The second includes counts of the number and lengths of root produced and deals only with the responses of those cuttings that actually did produce roots. This type of observation affords definite information, even if an easily rooted plant is employed.

There is, apparently, some decrease in activity as the length of the side chain increases. Responses due to the hexoic acid are usually less marked than those obtained with the butyric or acetic acid. However, the main feature of the results is the greater activity of the acids with an even number of carbon atoms in the side chain. This fact is of particular interest in view of the more frequent occurrence in nature of fatty acids with an even number of carbon atoms. The alternating effect with increasing length of side chain has been mentioned with reference to the indolyl series as determined by the *Avena* test (4).

It is interesting to note that naphthyl hexoic acid has pronounced physiological activity with, apparently, less tendency to cause damage or shortening of the roots than occurs with indolylacetic or naphthyl acetic acids. In consequence, higher members of the naphthyl series having an even number of carbon atoms in the chain may be of value in the treatment of cuttings, particularly with plants in which susceptibility to damage is a serious hazard.

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NITRIFICATION UNDER AND AFTER ALFALFA, BROME, TIMOTHY, AND WESTERN RYE GRASS

II. SOIL MICROBIOLOGICAL ACTIVITY[1]

By J. D. Newton[2], F. A. Wyatt[3], V. Ignatieff[4], and
A. S. Ward[5]

Abstract

Soil microbiological activity was measured for eight seasons, 1927 to 1934, in order to study some underlying causes of the comparative effects of alfalfa, brome, timothy, and western rye grass on the yield and nitrogen content of succeeding wheat crops.

When previously fallowed soil was seeded to alfalfa and grasses, the moisture and nitrate content of the soil were reduced, and generally remained at a relatively low level until the sods were plowed up. In the drier seasons the nitrates were reduced to a very low level or disappeared entirely in the grass and alfalfa plots. The nitrate content of the alfalfa plot soils was generally greater than that of the grass plots, and the brome grass plots were generally lower in nitrates than the timothy and western rye grass plots. The wheat plot soils generally contained more nitrate than the grass and alfalfa plots, especially in the drier seasons. When the sods were plowed up, nitrates accumulated in the alfalfa plots to a greater extent than in the grass plots and to a lesser extent generally in the brome plots than in the timothy and western rye plots. The greater nitrate content of the soil under wheat following alfalfa was observed for a period of three or four years in separate sets of plots plowed up two years apart. The nitrate level of the soil under wheat had a tendency to drop in mid-summer, often reaching its lowest point in July. The fallow plot soils were always higher in moisture than any of the cropped plots at the end of each season, and higher in nitrates in the latter half of each season.

The concentration of water-soluble phosphorus was greatest in the surface soil and seemed to be slightly higher under alfalfa and grasses than under wheat, but the total concentration was small and there was no very definite seasonal trend.

The numbers of fungi and bacteria, as determined by the plate count method for five seasons, 1929 to 1933, did not fluctuate very much in certain plots and seasons, but fluctuated greatly in others. The greatest fluctuations in fungal counts were observed under the first crop of wheat following brome grass, and in bacterial counts also under the first crop of wheat following sods, in the relatively moist season of 1931. Plate counts of actinomycetes did not fluctuate very greatly during the one season in which they were determined. The numbers of fungi were generally higher in the alfalfa plots than in the grass plots, but the differences between the grasses were apparently insignificant. Under the first crop of wheat following sods, large Mucor colonies predominated in the alfalfa plot soil plates and the counts were relatively low. Brome grass plot soils gave by far the highest counts of fungi, which consisted mainly of small Penicillium colonies, under the first crop of wheat following sods in 1931. The differences between numbers of bacteria in the alfalfa and grass plots were not very significant. The moisture content of the surface soil fluctuated greatly during most of the seasons. There was evidence of correlation between fluctuations in bacterial numbers and moisture, especially in certain seasons, in all the cropped

soils. There was less evidence of such correlation in the case of fungi, except under the first crop of wheat following brome grass in 1931. Fallow soil, though normally higher in moisture content in the latter part of each season, did not differ significantly from the grass-cropped soils in counts of fungi and bacteria. Although surface samples usually gave the highest counts, the deeper soil samples (to a depth of three feet) gave fairly high counts of both fungi and bacteria. During the season of 1930, amoebae were determined by the dilution plate count method; more than 1,000 and less than 10,000 per gram were nearly always found in both cropped and fallow soils.

The total nitrogen content of the plot soils showed considerable variation (owing to random sampling) from year to year, but no definite trend downwards or upwards during this period of seven years. The surface soil in every case contained most nitrogen and the subsoil least.

Introduction

This paper deals with soil microbiological aspects of the crop sequence studies at Edmonton described in Part I (19), in which are compared the effects of three grasses and a legume on the yield and nitrogen content of succeeding wheat crops. Soil microbiological activity was measured in order to study some of the underlying causes of the effects produced.

A block of land, divided into three sub-blocks, was seeded without a nurse crop, to alfalfa (*Medicago sativa* L.), brome (*Bromus inermis* Levss.), timothy (*Phleum pratense* L.), and western rye grass (*Agropyron tenerum* Vasey), in 1927. These crops were sown in quadruplicate plots, according to the Latin square system, in each of the three sub-blocks. The crop sequence history of the three sub-blocks is shown in the following tabular summary taken from Part I:

	I	II	III
1926	Fallow	Fallow	Fallow
1927	Seeded	Seeded	Seeded
1928	Hay	Hay	Hay
1929	Wheat	Hay	Hay
1930	Wheat	Hay	Hay
1931	Wheat	Wheat	Hay
1932	Wheat	Wheat	Hay
1933	Wheat	Wheat	Wheat
1934	Wheat	Wheat	Wheat

The soil in these plots is a fairly uniform, deep, black loam, rich in organic matter, underlaid by a clay subsoil, and almost neutral in reaction; this soil has been described in earlier publications (34).

Soil microbiological activity, as represented by nitrification, was measured in all plots for the eight seasons 1927 to 1934, except that in 1933 this measurement was confined to Sub-block III. In 1931 the samples were analyzed for water-soluble phosphorus.

Measurements of soil microbiological activity as represented by plate counts of fungi and bacteria (including actinomycetes) were started in 1929 and continued for a period of five seasons. Plate counts of actinomycetes

alone were made in 1929, and in 1930 the numbers of protozoa (amoebae) were determined by the dilution method. A supplementary experiment was carried out in 1932 to obtain a better idea of the distribution of bacteria and fungi in the deeper soil layers.

Supplementary determinations of nitrification and microbial numbers in fallow plots in the same field were made for several years. These fallow plots were outside the experimental block of plots, and were therefore not strictly comparable with the other plots. Nevertheless, it was felt that it would be interesting to make a general comparison of the cropped plots with the very different conditions of the fallow plots.

Total nitrogen determinations were made once a year, on samples of surface, subsurface and subsoil taken for nitrate determinations, to see if any definite trend in total nitrogen content could be measured in the relatively short period of years during which this experiment was carried on.

Methods

Soil Samples

Soil samples for the nitrate determinations were obtained in the following manner: Three or four borings to the depth of $6\frac{2}{3}$ in. were taken from each plot for the surface samples, two borings of $6\frac{2}{3}$ to 20 in. for the subsurface samples, and one or two borings of 20 to 40 in. for the subsoil samples. Composite samples were then made up by thoroughly mixing together the samples from the quadruplicate plots. Thus each surface composite sample was made up of 12 to 16 borings, each subsurface composite of 8 borings, and each subsoil composite of 4 to 8 borings.

The plots were sampled monthly for nitrate determinations. The first samples were taken in May and the last in September. The moisture content of each composite sample of soil was also determined.

Soil samples for microbial counts were taken to a depth of $6\frac{2}{3}$ in., as a rule. Three or four borings were taken from each of the quadruplicate plots, and thus each composite sample was made up of 12 to 16 borings. The samples were placed in clean glass jars and brought to the laboratory, where each composite sample was thoroughly mixed, diluted, and plated as soon as possible. The moisture content of each composite sample of soil was determined, also. Each sampling was done at approximately the same time of the day. The plots were sampled twice a month usually from early May to the middle of September, or about ten times during the season.

Nitrate

The nitrate determinations were carried out by the phenoldisulphonic acid method as modified by Harper (9). The composite soil samples obtained in the field were dried immediately at a temperature of 60 to 70° C. to stop bacterial activity. Higher temperatures were avoided to prevent losses of nitrate by volatilization. The dry samples were ground coarsely and placed in sealers for analysis.

Water-soluble Phosphorus

The Deniges method as modified by Parker and Fudge (20) was used for the determination of water-soluble phosphorus. By this procedure very small quantities of phosphate can be measured with accuracy. A weakness of the method is that it calls for great precision in measuring out the reagents, which causes delay, whereas rapidity is essential in carrying out the determination, because the blue colour developed by reduction of the complex phospho-molybdic acid fades rapidly on exposure to air. A 50-cc. sample of clear solution obtained by filtering the water extract (5 water to 1 soil) through a Buchner funnel was evaporated to dryness and ignited. The residue was taken up with dilute hydrochloric acid and the phosphate thus brought into solution was determined.

Total Nitrogen

The total nitrogen was determined by the ordinary Kjeldahl-Gunning-Hibbard method.

Numbers of Micro-organisms

The nutrient agar cultural plate count method was used for determining numbers of bacteria and fungi. The soil samples were brought into the laboratory in glass sealers and plated with the least possible delay.

The medium used for bacterial counts (including actinomycetes) was a sodium caseinate or nutrose agar medium (8). This medium is easily prepared and gives a reaction of approximately pH 6.8 without special adjustment.

A peptone-glucose acid agar medium recommended by Waksman (8) was used for fungi counts. The reaction was adjusted to pH 3.8 to 4.0 with sulphuric acid. A possible disadvantage of this medium is that it promotes the rapid growth of the proteolytic fungus, Mucor, which in certain cases prevented the appearance of slower developing fungi, such as Penicillium.

For actinomycetes counts, Waksman's nitrate-sucrose agar medium was used (8). This medium has a reaction of approximately pH 7.0.

The amoebae counts were made by the dilution method described by Cutler (4). Sterilized and hardened nutrient agar plates were inoculated with various dilutions, kept moist with sterile water, and examined microscopically after two and four weeks of incubation for presence of amoebae.

A dilution of 1 : 100,000 was generally used for bacterial counts, and 1 : 1000 for fungal counts. The plates were incubated for six days at room temperature for bacterial counts, as a rule, seven or eight days for actinomycetes counts, and two to five days at room temperature for fungal counts.

. Results

NITRIFICATION

In Tables I to XVI and in Fig. 1 the weighted average percentage moisture content of the surface, subsurface, and subsoil taken together is shown, and the total nitrate nitrogen content of the soil to the same total depth (40 in.).

TABLE I
SOIL MOISTURE—PERCENTAGE (WEIGHTED AVERAGE) IN 40-IN. DEPTH—1927

Sub-block	Crop	June 15	July 15	Aug. 13	Sept. 11	Seasonal average
I	Alfalfa	29.6	31.5	24.4	26.4	28.0
	Brome	30.3	30.1	22.6	24.8	27.0
	Timothy	30.2	31.0	24.1	25.4	27.7
	Western rye	29.9	32.3	24.7	26.1	28.2
II	Alfalfa	29.7	33.5	26.7	28.6	29.6
	Brome	30.3	33.2	25.4	27.8	29.2
	Timothy	31.7	29.9	23.5	24.2	27.3
	Western rye	30.5	31.5	24.6	24.8	27.8
III	Alfalfa	31.1	33.5	26.5	27.0	29.5
	Brome	30.6	32.0	26.2	25.6	28.6
	Timothy	30.8	33.2	25.0	25.6	28.6
	Western rye	31.3	30.3	25.9	30.4	29.5

TABLE II
NITRATE NITROGEN—POUNDS PER ACRE IN 40-IN. DEPTH—1927

Sub-block	Crop	June 15	July 15	Aug. 13	Sept. 11	Seasonal average
I	Alfalfa	228.2	193.6	171.0	106.6	174.8
	Brome	180.2	198.2	118.4	102.8	149.9
	Timothy	234.2	209.2	139.0	125.9	177.0
	Western rye	191.6	202.0	133.6	94.2	155.3
II	Alfalfa	235.0	227.8	212.0	150.4	206.3
	Brome	234.4	201.2	175.8	127.0	184.6
	Timothy	239.6	189.2	138.2	108.8	168.9
	Western rye	290.2	202.6	168.2	142.2	200.8
III	Alfalfa	274.4	266.4	243.6	164.8	237.8
	Brome	321.0	231.6	185.2	128.8	216.6
	Timothy	278.0	233.3	194.8	166.0	218.0
	Western rye	319.8	235.8	210.8	137.0	225.8

TABLE III
SOIL MOISTURE—PERCENTAGE (WEIGHTED AVERAGE) IN 40-IN. DEPTH—1928

Sub-block	Crop	May 17	June 15	July 10	Aug. 16	Sept. 19	Seasonal average
I	Alfalfa[1]	22.7	21.4	25.5	21.4	22.4	22.7
	Brome[1]	20.7	19.9	–	22.8	22.0	21.3
	Timothy[1]	21.8	20.7	25.2	22.1	22.3	22.4
	Western rye[1]	21.5	20.0	24.3	23.4	22.8	22.4
II	Alfalfa	24.8	22.0	25.7	20.2	20.6	22.7
	Brome	22.2	20.7	–	19.3	19.7	20.5
	Timothy	20.6	19.5	23.7	20.4	18.1	20.5
	Western rye	20.9	19.8	24.0	20.0	19.4	20.8
III	Alfalfa	25.4	23.1	26.1	21.8	22.5	23.8
	Brome	21.4	22.6	–	20.6	20.5	21.3
	Timothy	23.5	22.0	26.5	20.7	21.1	22.8
	Western rye	23.0	22.8	24.1	21.8	20.6	22.5

[1] *Broken in July.*

TABLE IV

NITRATE NITROGEN—POUNDS PER ACRE IN 40-IN. DEPTH—1928

Sub-block	Crop	May 17	June 15	July 10	Aug. 16	Sept. 19	Seasonal average
I	Alfalfa[1]	47.0	44.4	41.0	82.8	160.4	75.1
	Brome[1]	45.8	32.6	–	35.6	102.2	54.0
	Timothy[1]	56.0	62.2	46.2	59.4	137.2	72.2
	Western rye[1]	135.4	46.4	57.8	84.0	131.2	91.0
II	Alfalfa	133.0	92.2	64.6	52.4	57.4	79.9
	Brome	77.2	58.8	–	40.0	52.4	57.1
	Timothy	105.0	88.4	94.0	52.4	58.2	79.6
	Western rye	112.6	100.4	66.8	67.6	55.2	80.5
III	Alfalfa	131.6	57.8	86.6	45.0	60.6	76.3
	Brome	85.0	41.4	–	68.0	50.4	61.2
	Timothy	166.0	102.8	109.4	81.8	59.6	103.9
	Western rye	101.2	79.0	50.7	86.2	62.6	75.9

[1] *Broken in July.*

TABLE V

SOIL MOISTURE—PERCENTAGE (WEIGHTED AVERAGE) IN 40-IN. DEPTH—1929

Sub-block	Crop	May 14	June 14	July 16	Aug. 16 and 27	Sept. 11	Seasonal average
I	Wheat[1] after alfalfa	20.5	15.6	19.0	18.0	13.2	17.3
	Wheat[1] after brome	20.4	13.5	18.0	15.5	13.2	16.1
	Wheat[1] after timothy	19.8	14.0	19.8	19.4	11.9	17.0
	Wheat[1] after western rye	20.8	13.9	18.0	18.2	10.1	16.2
II	Alfalfa	18.5	11.9	15.8	15.8	10.8	14.6
	Brome	16.0	11.0	15.1	17.3	10.7	14.0
	Timothy	17 1	11.8	18.5	15.8	11.9	15.0
	Western rye	18.0	11.8	17.2	14.2	12.1	14.7
III	Alfalfa	26.5	16.1	20.6	16.1	10.2	17.9
	Brome	24.0	13.9	18.9	15.7	14.9	17.5
	Timothy	23.6	15.1	18.5	17.7	14.8	17.9
	Western rye	24.0	13.2	19.1	16.1	16.2	17.7
	Summerfallow	21.4	14.6	21.9	18.3	21.2	19.5

[1] *First crop of wheat after sod.*

TABLE VI

NITRATE NITROGEN—POUNDS PER ACRE IN 40-IN. DEPTH—1929

Sub-block	Crop	May 14	June 14	July 16	Aug. 16 and 27	Sept. 11	Seasonal average
I	Wheat[1] after alfalfa	122.8	197.8	82.6	112.2	120.2	127.1
	Wheat[1] after brome	58.6	101.4	65.8	40.8	58.2	65.0
	Wheat[1] after timothy	138.0	133.0	57.4	96.0	127.2	110.3
	Wheat[1] after western rye	131.2	113.8	93.4	106.6	153.6	119.7
II	Alfalfa	41.8	33.0	17.4	17.6	8.0	23.6
	Brome	Trace	Trace	10.2	14.8	Trace	5.0
	Timothy	Trace	Trace	33.2	Trace	Trace	6.6
	Western rye	Trace	Trace	12.2	7.4	Trace	3.9
III	Alfalfa	33.0	Trace	28.0	13.8	7.4	16.4
	Brome	Trace	Trace	Trace	Trace	Trace	Trace
	Timothy	Trace	Trace	30.2	Trace	Trace	6.0
	Western rye	Trace	Trace	8.6	Trace	Trace	1.7
	Summerfallow	62.8	84.8	114.6	151.4	217.6	126.2

First crop of wheat after sod.

TABLE VII

SOIL MOISTURE—PERCENTAGE (WEIGHTED AVERAGE) IN 40-IN. DEPTH—1930

Sub-block	Crop	May 12	June 16	July 14	Aug. 22	Sept. 19	Seasonal average
I	Wheat[1] after alfalfa	18.5	19.5	14.9	14.2	17.1	16.8
	Wheat[1] after brome	18.7	19.5	14.5	14.1	17.3	16.8
	Wheat[1] after timothy	18.7	21.6	14.2	15.1	17.4	17.4
	Wheat[1] after western rye	18.7	19.3	14.2	8.8	16.7	15.5
II	Alfalfa[2]	18.5	17.1	11.7	11.5	16.6	15.1
	Brome[2]	16.3	18.5	11.5	12.3	21.2	16.0
	Timothy[2]	17.6	18.7	12.8	8.5	19.1	15.3
	Western rye[2]	15.9	16.2	11.3	9.3	17.5	14.0
III	Alfalfa	19.1	22.0	14.9	14.5	17.6	17.6
	Brome	18.1	21.7	14.9	11.2	18.0	16.8
	Timothy	19.2	20.7	14.2	11.4	18.1	16.7
	Western rye	18.3	20.8	16.5	11.0	16.2	16.6
	Summerfallow	20.4	26.3	20.8	11.4	22.7	20.3

[1] *Second crop of wheat after sod.*
[2] *Broken in July.*

TABLE VIII

NITRATE NITROGEN—POUNDS PER ACRE IN 40-INCH DEPTH—1930

Sub-block	Crop	May 12	June 16	July 14	Aug. 22	Sept. 19	Seasonal average
I	Wheat[1] after alfalfa	112.8	96.0	71.8	147.6	55.0	96.6
	Wheat[1] after brome	78.4	128.4	36.4	95.6	23.2	72.4
	Wheat[1] after timothy	108.4	94.6	33.2	130.4	36.4	80.6
	Wheat[1] after western rye	84.6	71.0	36.4	136.4	39.0	73.5
II	Alfalfa[2]	15.2	34.4	Trace	83.8	45.6	35.8
	Brome[2]	Trace	Trace	None	33.6	5.4	7.8
	Timothy[2]	Trace	Trace	None	81.6	14.2	19.2
	Western rye[2]	Trace	Trace	Trace	53.4	22.2	15.1
III	Alfalfa	11.0	13.2	Trace	23.6	Trace	9.6
	Brome	Trace	Trace	None	Trace	Trace	Trace
	Timothy	Trace	41.4	Trace	55.4	Trace	19.4
	Western rye	Trace	Trace	Trace	35.0	Trace	7.0
	Summerfallow	71.0	42.8	110.0	192.4	60.4	95.3

[1] *Second crop of wheat after sod.*

[2] *Broken in July.*

TABLE IX

SOIL MOISTURE—PERCENTAGE (WEIGHTED AVERAGE) IN 40-IN. DEPTH—1931

Sub-block	Crop	May 13	June 25	July 24	Aug. 18	Sept. 15 and 25	Seasonal average
I	Wheat[1] after alfalfa	14.7	18.4	19.0	19.4	19.6	18.2
	Wheat[1] after brome	15.6	17.9	18.8	20.3	19.5	18.4
	Wheat[1] after timothy	12.9	19.0	18.1	21.1	17.9	17.8
	Wheat[1] after western rye	14.3	19.5	15.9	20.0	18.6	17.7
II	Wheat[2] after alfalfa	13.5	18.5	18.0	18.7	17.0	17.1
	Wheat[2] after brome	13.0	18.5	19.8	20.5	20.3	18.4
	Wheat[2] after timothy	12.5	20.4	18.3	20.1	18.6	18.0
	Wheat[2] after western rye	11.7	17.8	18.0	20.9	19.0	17.5
III	Alfalfa	12.9	18.6	19.9	23.6	16.4	18.3
	Brome	10.6	17.6	20.2	23.7	20.7	18.6
	Timothy	13.2	21.4	23.7	24.9	21.2	20.9
	Western rye	13.4	20.0	22.5	26.1	21.9	20.8
	Summerfallow	15.0	17.1	23.8	24.6	24.4	21.0

[1] *Third crop of wheat after sod.*

[2] *First crop of wheat after sod.*

TABLE X

NITRATE NITROGEN—POUNDS PER ACRE IN 40-IN. DEPTH—1931

Sub-block	Crop	May 13	June 25	July 24	Aug. 18	Sept. 15 and 25	Seasonal average
I	Wheat[1] after alfalfa	182.8	116.4	73.4	7.6	64.0	88.8
	Wheat[1] after brome	94.2	91.8	38.4	7.2	17.2	49.8
	Wheat[1] after timothy	139.6	108.0	61.4	8.6	45.8	72.7
	Wheat[1] after western rye	151.2	109.0	55.4	8.4	63.2	77.4
II	Wheat[2] after alfalfa	138.2	99.6	45.6	31.2	43.0	71.5
	Wheat[2] after brome	66.4	26.2	23.0	9.4	15.4	28.1
	Wheat[2] after timothy	122.8	94.2	50.4	9.8	40.4	63.5
	Wheat[2] after western rye	139.4	62.8	33.8	9.8	58.4	60.8
III	Alfalfa	51.8	14.6	34.2	21.2	17.2	27.8
	Brome	14.8	17.8	28.2	14.6	17.2	18.5
	Timothy	116.6	34.4	46.4	15.8	13.8	45.4
	Western rye	57.6	97.6	67.8	40.2	61.6	65.0
	Summerfallow	242.2	282.6	214.6	276.8	273.2	257.9

[1] Third crop of wheat after sod.
[2] First crop of wheat after sod.

TABLE XI

SOIL MOISTURE—PERCENTAGE (WEIGHTED AVERAGE) IN 40-IN. DEPTH—1932

Sub-block	Crop	May 17	June 17	July 17	Aug. 17	Sept. 17	Seasonal average
I	Wheat[1] after alfalfa	22.7	22.8	19.2	18.7	17.6	20.5
	Wheat[1] after brome	23.3	23.4	17.4	15.5	14.0	18.7
	Wheat[1] after timothy	19.1	20.7	16.3	13.2	13.9	16.6
	Wheat[1] after western rye	21.0	20.3	19.0	13.5	15.3	17.8
II	Wheat[2] after alfalfa	22.2	19.8	18.7	13.4	15.2	17.9
	Wheat[2] after brome	24.1	21.3	16.9	14.3	15.0	18.3
	Wheat[2] after timothy	21.2	22.7	15.8	14.2	13.0	17.4
	Wheat[2] after western rye	21.1	21.2	13.3	11.9	11.4	15.8
III	Alfalfa[3]	20.4	17.1	14.0	12.0	14.1	15.5
	Brome[3]	21.0	20.3	20.5	15.3	18.4	19.1
	Timothy[3]	21.3	20.0	19.1	15.7	18.1	18.8
	Western rye[3][4]	23.2	20.2	22.0	16.7	19.1	20.2
	Summerfallow	21.7	23.5	26.5	20.1	23.7	23.1

[1] Fourth crop of wheat after sod.
[2] Second crop of wheat after sod.
[3] Broken in July.
[4] Killed by "take-all".

TABLE XII

NITRATE NITROGEN—POUNDS PER ACRE IN 40-IN. DEPTH—1932

Sub-block	Crop	May 17	June 17	July 17	Aug. 17	Sept. 17	Seasonal average
I	Wheat[1] after alfalfa	57.3	55.6	44.8	5.2	49.6	42.5
	Wheat[1] after brome	72.0	37.7	9.0	7.2	47.8	34.7
	Wheat[1] after timothy	70.2	64.1	6.6	5.2	45.2	38.4
	Wheat[1] after western rye	52.9	57.9	7.2	5.4	42.1	33.1
II	Wheat[2] after alfalfa	65.2	53.6	89.0	50.8	57.2	63.1
	Wheat[2] after brome	34.3	61.2	42.1	7.8	25.8	34.2
	Wheat[2] after timothy	41.5	63.5	42.6	20.0	22.0	37.9
	Wheat[2] after western rye	39.5	79.2	44.8	7.2	33.4	40.8
III	Alfalfa[3]	20.4	17.6	11.4	17.4	56.4	24.6
	Brome[3]	9.9	9.0	11.6	15.0	33.6	15.8
	Timothy[3]	8.3	27.4	44.2	3.3	40.6	24.7
	Western rye[3][4]	38.5	68.7	42.2	35.4	61.2	49.2
	Summerfallow	47.1	92.1	115.6	125.8	136.2	103.3

[1] *Fourth crop of wheat after sod.*
[2] *Second crop of wheat after sod.*
[3] *Broken in July.*
[4] *Killed by "take-all".*

TABLE XIII

SOIL MOISTURE—PERCENTAGE (WEIGHTED AVERAGE) IN 40-IN. DEPTH—1933

Sub-block	Crop	May 11	June 12	Aug. 16	Seasonal average
III	Wheat[1] after alfalfa	20.9	18.0	11.7	16.9
	Wheat[1] after brome	21.6	18.7	13.2	17.8
	Wheat[1] after timothy	23.7	22.7	14.1	20.2
	Summerfallow	18.2	23.8	20.1	20.7

[1] *First crop of wheat after sod.*

TABLE XIV

NITRATE NITROGEN—POUNDS PER ACRE IN 40-IN. DEPTH—1933

Sub-block	Crop	May 11	June 12	Aug. 16	Seasonal average
III	Wheat[1] after alfalfa	74.6	111.8	39.4	75.3
	Wheat[1] after brome	75.2	96.0	Trace	57.1
	Wheat[1] after timothy	60.0	46.8	Trace	35.6
	Summerfallow	130.0	102.8	194.0	142.3

[1] *First crop of wheat after sod.*

TABLE XV

SOIL MOISTURE—PERCENTAGE (WEIGHTED AVERAGE) IN 40-IN. DEPTH—1934

Sub-block	Crop	May 7	June 8	July 18	Aug. 10	Sept. 7	Seasonal average
1	Wheat[1] after alfalfa	17.7	19.5	16.3	15.5	15.8	17.0
	Wheat[1] after brome	17.3	18.6	15.4	14.8	15.0	16.2
	Wheat[1] after timothy	16.6	16.7	15.5	14.0	14.0	15.4
	Wheat[1] after western rye	16.8	23.1	15.3	14.6	14.5	16.9
II	Wheat[2] after alfalfa	17.1	17.3	16.7	14.4	14.2	15.9
	Wheat[2] after brome	17.6	18.1	15.6	15.1	13.9	16.1
	Wheat[2] after timothy	17.3	18.5	16.6	15.5	13.9	16.4
	Wheat[2] after western rye	17.3	18.1	16.3	14.7	13.9	16.1
III	Wheat[3] after alfalfa	18.1	18.3	17.0	16.1	15.1	16.9
	Wheat[3] after brome	18.1	20.3	18.8	16.1	15.4	17.7
	Wheat[3] after timothy	19.6	20.3	18.1	16.6	15.7	18.1

[1] *Sixth crop of wheat after sod.*
[2] *Fourth crop of wheat after sod.*
[3] *Second crop of wheat after sod.*

TABLE XVI

NITRATE NITROGEN—POUNDS PER ACRE IN 40-IN. DEPTH—1934

Sub-block	Crop	May 7	June 8	July 18	Aug. 10	Sept. 7	Seasonal average
I	Wheat[1] after alfalfa	45.0	44.6	7.6	68.2	41.0	41.3
	Wheat[1] after brome	59.4	40.2	7.4	54.0	39.4	40.1
	Wheat[1] after timothy	41.6	51.8	Trace	62.8	36.4	38.5
	Wheat[1] after western rye	42.6	56.2	Trace	60.4	40.0	39.8
II	Wheat[2] after alfalfa	89.8	81.0	13.6	87.2	83.2	71.0
	Wheat[2] after brome	54.2	62.4	Trace	68.2	28.4	42.6
	Wheat[2] after timothy	46.2	53.4	Trace	66.0	46.2	42.4
	Wheat[2] after western rye	48.4	51.4	Trace	59.0	43.0	40.4
III	Wheat[3] after alfalfa	91.0	77.8	22.4	81.8	72.8	69.2
	Wheat[3] after brome	47.8	40.0	Trace	64.4	12.0	32.8
	Wheat[3] after timothy	59.6	36.2	24.4	61.2	11.8	38.6

[1] *Sixth crop of wheat after sod.*
[2] *Fourth crop of wheat after sod.*
[3] *Second crop of wheat after sod.*

The nitrate nitrogen is expressed as pounds per acre for convenient comparison of the amount present with the amount required per acre by various crops.

It would require too much space to tabulate the corresponding data for each crop and soil depth separately, but examples illustrating the depth distribution under alfalfa and timothy sods and wheat following these sods, and in fallow soil, are shown in Table XVII. The moisture content is expressed as percentage of water-free soil, and the nitrate content as parts of nitrate nitrogen per million parts of water-free soil.

TABLE XVII

MOISTURE AND NITRATE NITROGEN IN SURFACE, SUBSURFACE, AND SUBSOIL. SEASONAL AVERAGES AND AVERAGE OF SEASONS

Sub-block	Depth, in.	1927 Moist. %	1927 Nitrate N, p.p.m.	1928 Moist. %	1928 Nitrate N, p.p.m.	1929 Moist. %	1929 Nitrate N, p.p.m.	1930 Moist. %	1930 Nitrate N, p.p.m.	1931 Moist. %	1931 Nitrate N, p.p.m.	1932 Moist. %	1932 Nitrate N, p.p.m.	Average Moist. %	Average Nitrate N, p.p.m.
							Alfalfa 1927–28, and Wheat 1929–32								
I	0–6¾	37.1	21.0	32.2	15.5	23.0	32.1	19.0	23.6	26.6	14.0	24.5	6.6	27.1	18.8
	6¾–20	26.8	15.2	21.4	4.1	15.6	12.9	14.9	12.4	15.7	9.7	18.2	3.2	18.8	9.6
	20–40	25.7	12.0	20.3	4.6	16.4	1.9	17.4	0.0	17.0	3.7	20.1	2.7	19.5	4.1
							Timothy 1927–28, and Wheat 1929–32								
I	0–6¾	36.8	17.2	30.6	9.4	23.8	22.0	19.9	15.2	24.8	11.7	19.3	6.0	25.9	13.6
	6¾–20	26.1	17.0	21.3	4.5	15.0	10.5	14.5	7.4	16.6	6.2	16.6	3.1	18.3	8.1
	20–40	25.7	12.4	20.5	5.9	16.0	4.0	18.5	3.4	16.3	4.0	15.7	2.6	18.8	5.4
							Alfalfa 1927–32								
III	0–6¾	38.8	25.4	31.5	6.1	21.6	5.0	21.7	4.8	25.2	10.5	18.8	5.8	26.3	9.6
	6¾–20	29.3	22.4	23.1	6.5	20.2	1.6	17.3	0.0	18.9	1.7	16.2	2.1	20.8	5.7
	20–40	26.6	16.1	21.6	6.4	15.1	0.0	16.6	0.0	15.6	0.0	13.7	0.7	18.2	3.9
							Timothy 1927–32								
III	0–6¾	35.7	20.2	26.6	5.1	20.2	1.3	17.5	3.2	27.3	7.7	21.3	5.8	24.8	7.2
	6¾–20	28.5	22.4	21.6	9.4	17.3	0.9	14.8	1.0	21.1	1.4	19.1	1.7	20.4	6.1
	20–40	26.4	14.6	22.3	9.3	17.6	0.0	17.7	1.5	18.6	4.0	17.8	1.6	20.1	5.2
							Fallow 1929–32								
—	0–6¾					30.7	23.6	26.5	28.2	28.8	33.4	31.5	23.7	29.4	27.2
	6¾–20					19.0	9.5	18.7	6.7	21.1	27.9	25.0	6.8	20.9	12.7
	20–40					16.0	6.8	19.3	2.0	18.3	13.3	19.1	4.1	18.2	6.5
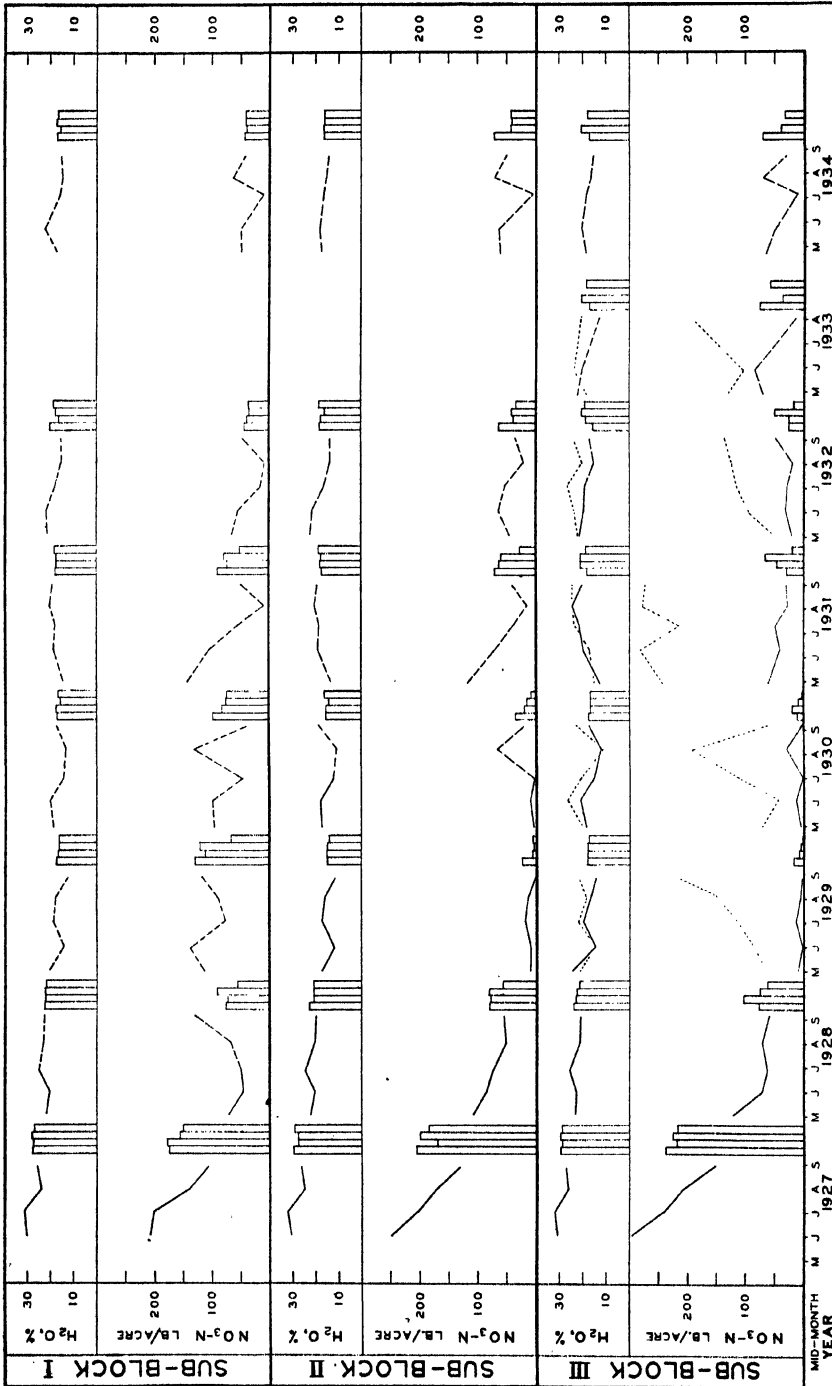
FIG. 1. *Seasonal fluctuations in moisture and nitrate nitrogen to a depth of 40 in. under hay crops (solid lines), under succeeding wheat crops (broken lines), and under fallow (dotted lines). Mean seasonal values under alfalfa, timothy, western rye and brome (in that order from left to right) and under wheat following these sods, shown by histograms.*

The graphs (Fig. 1) show that the moisture and nitrate content of the soil in all three sub-blocks were relatively high in the fallow soil at the beginning of the experiment. After the plots were seeded to alfalfa and grasses in 1927, the moisture and nitrate content of the soil decreased.

Wheat was first grown on Sub-block I in 1929, and the nitrate content of the soil under wheat was for several years generally relatively high following alfalfa, and relatively low following brome grass. Even under the third crop of wheat (1931) following alfalfa, brome, timothy, and western rye grass, the nitrates were generally highest following alfalfa and lowest following brome grass. Afterwards, in 1932 and 1934, the differences were apparently insignificant. The nitrate level of the soil under wheat has a tendency to drop in mid-summer, often reaching its lowest point in July, as shown in the tables and Fig. 1. There was no corresponding decrease in soil moisture, the drop corresponding rather to the period of maximum absorption of nitrates by the growing crop.

After the Sub-block II plots were seeded to alfalfa and grasses in 1927, the nitrate level dropped from year to year, reaching quite a low level in 1929 and 1930, much lower than that of the wheat plots of Sub-block I. After the plots were plowed in 1930 the nitrates increased.

A comparison of the nitrate content of the soil under alfalfa and the different grass sods, from 1927 to 1930, shows that nitrates were generally highest under alfalfa. The tables show that nitrates were highest in the alfalfa plots in three out of four monthly determinations in 1927; in four out of five determinations in 1929; and in the early part of the 1930 season, before the plots were plowed. However, in 1928 the differences between the alfalfa, timothy, and western rye grass plots were apparently insignificant. In three out of the five monthly determinations of the 1929 season there were measurable quantities of nitrate under alfalfa and none or only traces under the grasses. Similarly in May and June, 1930, before the sods were plowed, there were measurable quantities of nitrate in the alfalfa plot soils, but none or only traces in the grass plot soils.

After the sods were broken in 1930 the nitrate content of all plots increased, as shown in Fig. 1. The tables and Fig. 1 histograms show that the nitrate level was generally highest under wheat following alfalfa. Even in 1934 under the fourth crop of wheat, the nitrate level was highest following alfalfa. In the latter part of the 1930 season after the sods were plowed, and throughout the 1931 season when the plots were under wheat, the nitrate level was lower following brome than following timothy or western rye grass. These results confirm the results obtained in Sub-block I.

In Sub-block III, after the plots were seeded to alfalfa and grasses in 1927, the nitrate level dropped from year to year, reaching, as in Sub-block II, quite a low level in 1929 and 1930, much lower than the wheat plot soils of Sub-block I. Although the Sub-block III plots were still under alfalfa and grasses in 1931 and 1932, the nitrate level was generally higher than in 1929 and 1930, and this higher level is attributed to moister seasons. The nitrate content of the soil under wheat in 1933 and 1934 was quite variable.

A comparison of the nitrate content of the soil under alfalfa and grass, from 1927 to 1932, shows that the alfalfa plots were not highest in nitrates as commonly as they were in Sub-block II. However, in 1927 and 1929 the alfalfa plots were generally highest in this respect, and throughout the period from 1927 to 1932 the brome grass plots were generally lower in nitrates than the alfalfa, timothy, and western rye grass plots, as shown in the tables and Fig. 1 histograms. In 1929 and again in 1930 there was never more than a trace of nitrate present in the brome grass plots at the time of the monthly determinations.

After the sods were broken in 1932, the nitrate content of all plots increased to some extent. Under the first and second crops of wheat, in 1933 and 1934, the nitrates were highest following alfalfa, but not consistently lowest following brome grass. No results are given for the wheat plots following western rye grass because these plots were badly infected by root rot before they were plowed in 1932.

As previously noted, the main experiment was supplemented by determinations of moisture and nitrates in nearby fallow plots for the five seasons, 1929 to 1933, to compare these specially favourable conditions with those of the cropped soils. A different fallow plot within the same field was sampled each year. The results are shown in Tables V to XIV and Fig. 1. These data show that the moisture content of the soil was higher in the fallow plots at the end of each of the five seasons than in any of the cropped plots. The nitrates, also, were nearly always higher in the fallow plots than in the cropped plots, and always higher in the latter half of each season.

WATER-SOLUBLE PHOSPHORUS

There has been much controversy on the subject of determination of available phosphorus, that is, of phosphorus present in the soil that could be readily utilized by plants. Methods of determining available phosphorus have been evolved by Dyer (7), Neubauer and Schneider (16), Winogradsky (31), Truog (26) and others. Neubauer makes use of living plants for the determination. Winogradsky evolved the Azotobacter method. Other investigators employ the method of extraction of soil with different types of solvents which are supposed to simulate the action of roots. Much can be said for these methods, but one must always bear in mind that little as yet is known about the actual form of phosphorus absorbed, and as to how plants extract it from the·soil. Any method employed is therefore merely empirical.

In this experiment it was decided to use distilled water for making soil extracts, because it was thought that, whatever the effect of the plants on the soil or its solution, the phosphorus that was present in a water extract would be the most readily available to the plants.

Ignition of the substances obtained by extraction of the soil was carried out to remove the organic matter which would otherwise interfere with the blue colour developed in the determination (20), giving it a yellow tinge. On ignition, however, the organic phosphorus present was changed to the

TABLE XVIII

WATER-SOLUBLE PHOSPHORUS AS PO4 (P.P.M.) IN SURFACE, SUBSURFACE, AND SUBSOIL—1931

Sub-block	Crop	Depth, in.	May 13	June 25	July 24	Aug. 18	Sept. 15, 25	Horizontal averages	Vertical averages
I	Wheat[1] after timothy	0 – 6⅔	8.1	8.3	9.6	8.9	5.4	8.1	
	Wheat[1] after timothy	6⅔ – 20	3.5	3.7	3.2	4.1	2.8	3.5	4.0
	Wheat[1] after timothy	20 – 40	0.6	0.3	0.2	0.3	0.5	0.4	
I	Wheat[1] after alfalfa	0 – 6⅔	9.3	10.2	10.5	9.9	6.7	9.3	
	Wheat[1] after alfalfa	6⅔ – 20	4.3	3.7	3.1	3.5	2.5	5.4	4.4
	Wheat[1] after alfalfa	20 – 40	0.8	0.6	—	0.2	0.6	0.5	
I	Wheat[1] after brome	0 – 6⅔	9.6	8.1	8.7	10.3	5.3	8.4	
	Wheat[1] after brome	6⅔ – 20	3.6	4.3	3.8	4.1	2.6	3.7	4.3
	Wheat[1] after brome	20 – 40	1.8	0.7	0.2	0.2	0.8	0.7	
I	Wheat[1] after western rye	0 – 6⅔	9.3	9.9	8.7	10.6	7.8	9.2	
	Wheat[1] after western rye	6⅔ – 20	4.1	3.7	3.6	4.8	3.0	3.8	4.5
	Wheat[1] after western rye	20 – 40	0.7	0.4	0.3	0.1	0.5	0.4	
II	Wheat[2] after alfalfa	0 – 6⅔	6.6	9.7	10.6	12.1	5.7	8.9	
	Wheat[2] after alfalfa	6⅔ – 20	3.9	4.5	2.4	—	3.5	3.6	4.3
	Wheat[2] after alfalfa	20 – 40	0.6	0.4	0.3	0.2	0.9	0.5	
II	Wheat[2] after brome	0 – 6⅔	10.2	9.2	10.9	11.9	7.7	10.0	
	Wheat[2] after brome	6⅔ – 20	3.7	4.6	4.5	4.6	3.3	4.1	4.9
	Wheat[2] after brome	20 – 40	1.2	0.5	0.4	0.2	1.0	0.7	
II	Wheat[2] after timothy	0 – 6⅔	8.3	10.1	10.6	9.1	5.8	8.8	
	Wheat[2] after timothy	6⅔ – 20	4.5	5.2	4.0	6.2	4.1	4.8	4.7
	Wheat[2] after timothy	20 – 40	0.5	0.2	0.5	0.7	0.0	0.4	
II	Wheat[2] after western rye	0 – 6⅔	7.6	9.7	9.5	9.5	5.3	8.3	
	Wheat[2] after western rye	6⅔ – 20	3.7	3.2	3.7	4.3	2.6	3.5	4.1
	Wheat[2] after western rye	20 – 40	0.6	0.4	0.6	0.2	1.1	0.6	

[1] Third crop of wheat after breaking.
[2] First crop of wheat after breaking.

TABLE XVIII—*Concluded*

WATER-SOLUBLE PHOSPHORUS AS PO_4 (P.P.M.) IN SURFACE, SUBSURFACE, AND SUBSOIL—1931—*Concluded*

Sub-block	Crop	Depth, in.	May 13	June 25	July 24	Aug. 18	Sept. 15, 25	Horizontal averages	Vertical averages
III	Timothy	0 – 6⅔	9.0	11.8	11.9	12.2	8.1	10.6	
	Timothy	6⅔ – 20	3.9	4.4	4.7	3.9	2.8	3.9	5.0
	Timothy	20 – 40	0.8	0.5	0.9	0.1	0.5	0.6	
III	Alfalfa	0 – 6⅔	10.0	14.3	12.4	13.5	9.5	11.9	
	Alfalfa	6⅔ – 20	4.1	3.9	4.4	6.0	3.5	4.4	5.7
	Alfalfa	20 – 40	0.9	0.4	0.8	0.7	0.6	0.7	
III	Brome	0 – 6⅔	10.9	11.9	12.2	13.5	8.2	11.3	
	Brome	6⅔ – 20	4.9	4.0	3.7	3.9	3.5	4.0	5.4
	Brome	20 – 40	1.0	0.7	0.8	0.6	1.3	0.9	
III	Western rye	0 – 6⅔	7.9	12.0	11.2	12.8	8.9	10.6	
	Western rye	6⅔ – 20	5.9	4.1	3.4	3.1	3.5	4.0	5.1
	Western rye	20 – 40	1.7	0.5	0.6	0.6	0.5	0.8	
III	Fallow	0 – 6⅔	10.4	11.7	11.2	12.6	10.6	11.3	
	Fallow	6⅔ – 20	3.5	2.5	3.2	3.0	1.9	2.8	4.8
	Fallow	20 – 40	0.6	0.3	0.0	0.0	1.0	0.4	

phosphate form and determined as such. A criticism of this procedure is that the plants may not be able to utilize organic phosphorus, and thus the figures may be of little value. However, Whiting and Heck with their work on phytin (28), and others, have shown that plants can make very good use of certain forms of organic phosphorus, directly or indirectly.

The data obtained during the season of 1931 are given in Table XVIII. In considering the results of this season's work one notices that the greatest amount of water-soluble phosphorus is present in the upper six inches of soil, with a gradual decrease downwards. In fact, in the subsoil there is very little water-soluble phosphorus present. It was possible to test the water extracts of a few subsoils directly without first evaporating them and igniting the organic matter. No phosphate was detected in such solutions before ignition, showing that all the water-soluble phosphorus in the subsoil was present in the organic form.

There is no great difference between the figures of the individual plots. However, the concentration of the phosphate in the top layer of the soil seems to be slightly higher under grasses, and in particular under alfalfa, than under wheat, as shown in the vertical average column of Table XVIII. Furthermore, during the actual determination, differences in the intensity of the blue colour developed were clearly seen. Since, however, the differences are slight and are almost within the range of experimental error, one must avoid any very definite conclusion on this point.

There does not seem to be any definite fluctuation in water-soluble phosphorus during the growing season, but the figures show a decrease in the month of September. Only further work can show whether this is a regular phenomenon, or merely peculiar to this season.

MICROBIAL NUMBERS

The seasonal curves for moisture and numbers of micro-organisms in the surface soil are shown in Fig. 2, and the detailed results in Tables XIX to XXIII.

The numbers of micro-organisms in the Sub-block I plots were determined in 1929 and 1930, under the first and second crops of wheat following alfalfa, timothy, and western rye grass.

The curves show that the moisture content of the surface soil fluctuated greatly in both 1929 and 1930. The numbers of fungi fluctuated but little, and the differences between the different plots are apparently insignificant. The numbers varied from about 10 to 30 thousands per gram. It was observed that large Mucor colonies predominated on the plates inoculated with soil from the alfalfa plots, but not on the other plates (Fig. 3). The numbers of bacteria fluctuated more than the numbers of fungi, and there is some evidence of a correlation between fluctuations in bacterial numbers and moisture, but it is doubtful if the differences between the bacterial numbers in the different plots are significant. The numbers varied from about 4 to 12 millions per gram. The numbers of actinomycetes did not fluctuate very

much until the later part of the season. Through most of the season they varied between one and two millions per gram.
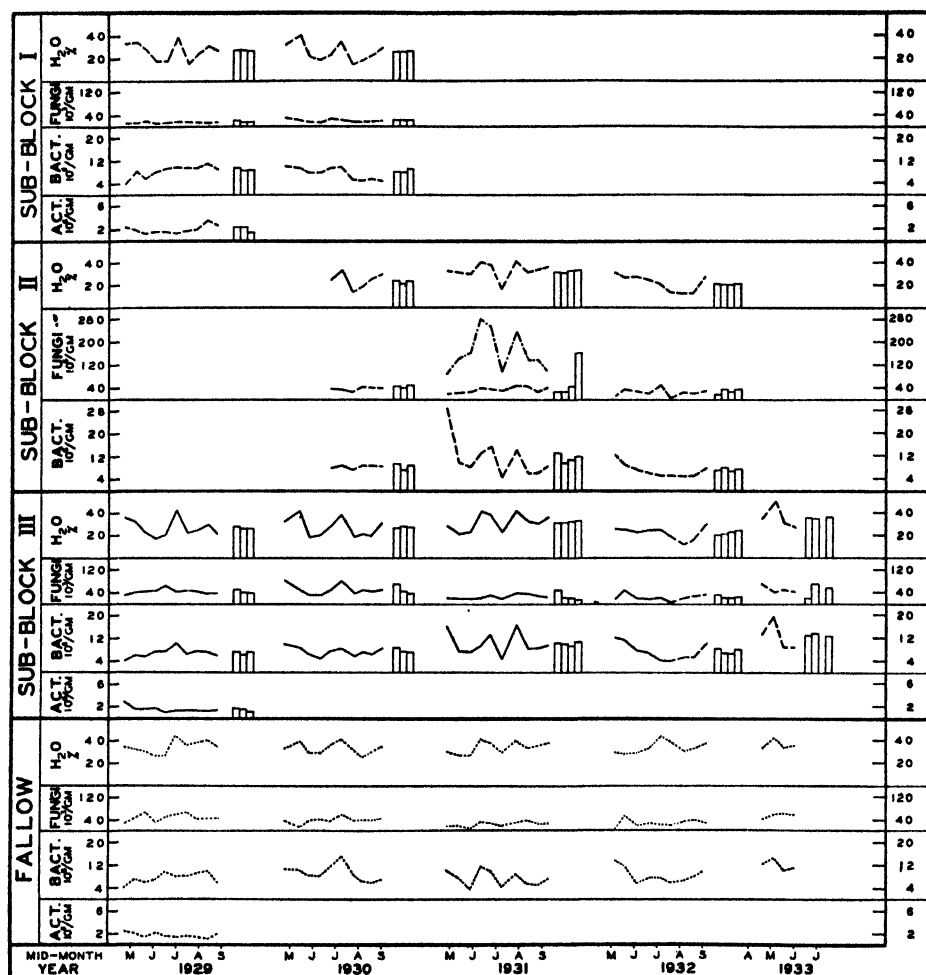
FIG. 2. *Seasonal fluctuations in moisture content and number of fungi, bacteria and actinomycetes in upper 6⅔ in. of soil under hay crops (solid lines), under succeeding wheat crops (broken lines), and under fallow (dotted lines). Fungi in brome plots in Sub-block II, 1931, graphed separately (upper line). Mean seasonal values under alfalfa, timothy, western rye and brome (in that order from left to right) and under wheat following these sods, shown by histograms.*

The numbers of micro-organisms in the alfalfa, timothy, and western rye grass plots of Sub-block II were determined in early July, 1930, just before the sods were plowed up, and throughout the remainder of that season. The numbers under the first and second crops of wheat following alfalfa, brome, timothy, and western rye were determined in 1931 and 1932.

TABLE XIX

SEASONAL FLUCTUATIONS IN MOISTURE AND NUMBERS OF MICRO-ORGANISMS IN UPPER 6⅔ IN. OF SOIL, UNDER ALFALFA, GRASSES, AND FALLOW, IN 1929

Sub-block	Crop	May 6	May 20	June 3	June 17	July 1	July 15	July 29	Aug. 12	Aug. 26	Sept. 9	Average
						Moisture, %						
I	Wheat[1] after alfalfa	34.2	34.7	28.7	17.6	20.2	40.4	16.6	26.6	32.0	27.4	27.8
	Wheat[1] after timothy	34.2	36.0	29.5	19.0	18.7	39.4	16.6	24.1	31.6	27.9	27.7
	Wheat[1] after western rye					18.7	39.7	16.1	24.5	33.3	28.1	26.7
III	Alfalfa	36.1	34.6	25.5	19.2	22.5	42.7	20.3	25.5	29.4	21.6	27.7
	Timothy	35.6	30.9	20.8	15.3	20.8	43.3	23.4	24.3	30.0	20.8	26.5
	Western rye					17.9	41.5	23.7	24.9	29.1	21.4	26.4
A-1	Fallow	33.9	31.8	29.9	26.4	25.9	44.0	36.2	38.2	40.4	34.0	34.1
						Bacteria, millions per gm.						
I	Wheat[1] after alfalfa	4.24	8.08	9.41	9.91	9.81	10.67	11.22	10.45	11.22	12.10	9.71
	Wheat[1] after timothy	4.24	9.65	3.07	7.17	10.99	11.02	10.19	9.42	10.95	8.38	8.51
	Wheat[1] after western rye					7.92	8.08	8.18	8.56	12.80	7.52	8.84
III	Alfalfa	5.00	5.65	5.64	8.86	8.11	14.34	5.85	6.81	6.65	5.46	7.24
	Timothy	2.88	6.23	5.64	5.87	7.75	10.40	6.60	7.84	8.01	6.28	6.75
	Western rye					6.86	6.71	7.31	8.11	7.66	6.97	7.27
A-1	Fallow	4.13	7.06	6.01	7.06	9.62	8.27	8.36	9.39	9.87	5.62	7.55
						Fungi, thousands per gm.						
I	Wheat[1] after alfalfa	15	15	21	21	28	24	23	21	21	22	21
	Wheat[1] after timothy	15	16	24	9	15	19	14	18	10	16	16
	Wheat[1] after western rye					12	16	23	15	16	17	16
III	Alfalfa	33	43	54	41	89	52	70	54	53	62	55
	Timothy	27	43	35	53	69	33	31	44	36	25	40
	Western rye					40	47	44	41	22	28	37
A-1	Fallow	27	48	68	33	49	58	66	45	46	46	49
						Actinomycetes, millions per gm.						
I	Wheat[1] after alfalfa	2.66	1.24	1.24	1.52		1.48	1.74	2.25	5.70	2.21	2.23
	Wheat[1] after timothy	2.18	2.56	1.36	1.62		1.24	1.84	1.94	4.60	3.77	2.34
	Wheat[1] after western rye					1.57	1.67	1.68		0.50	2.14	1.51
III	Alfalfa	2.47	1.84	1.89	1.96	1.07	2.09	1.89	1.43	1.48	1.15	1.73
	Timothy	3.25	1.55	1.31	1.50	0.96	1.22	1.46	1.33	1.87	1.39	1.65
	Western rye						0.62	1.30	1.34	0.48	1.78	1.08
A-1	Fallow	2.36	2.08	1.44	2.13	1.60	1.44	1.58	1.38	1.11	1.95	1.71

TABLE XX

SEASONAL FLUCTUATIONS IN MOISTURE, AND NUMBERS OF MICRO-ORGANISMS IN UPPER 6¾ IN. OF SOIL, UNDER ALFALFA, GRASSES, AND FALLOW, IN 1930

Sub-block	Crop	May 7	May 27	June 10	June 24	July 8	July 22	Aug. 6	Aug. 19	Sept. 2	Sept. 15	Average
	Moisture, %											
I	Wheat[1] following alfalfa	33.0	40.5	20.8	19.1	22.7	35.7	14.8	18.4	22.8	30.3	25.8
	Wheat[1] following timothy	32.9	41.8	20.7	18.6	24.6	35.9	15.1	18.5	24.2	31.9	25.8
	Wheat[1] following western rye	32.7	41.3	23.2	20.0	25.9	34.4	14.3	18.9	22.0	29.9	26.3
II	Alfalfa[2]	30.5	39.4	18.9	20.2	23.6	36.7	17.2	18.3	26.5	29.6	25.3
	Timothy[2]	33.0	45.4	18.3	20.5	26.7	26.4	9.9	18.6	24.3	29.5	22.6
	Western rye[2]	33.2	40.7	17.4	18.5	26.0	30.9	16.1	17.6	26.6	30.3	24.6
III	Alfalfa					25.5	38.5	17.2	20.9	19.9	29.2	26.0
	Timothy					30.2	39.8	17.7	22.5	19.7	30.9	27.7
	Western rye					28.1	37.8	21.4	21.2	21.2	31.4	27.1
B-5	Fallow	33.7	41.9	29.5	28.8	35.8	40.9	31.2	24.8	29.9	34.7	33.1
B-5	Fallow (variability)	32.7	37.2	28.6	28.3			30.7			34.3	32.0
	Bacteria, millions per gm.											
I	Wheat[1] following alfalfa	10.16	9.62	10.20	8.68	9.26	11.48	5.33	6.07	6.07	5.62	8.25
	Wheat[1] following timothy	11.00	10.56	7.10	6.76	12.12	10.61	7.05	3.74	5.33	5.50	7.98
	Wheat[1] following western rye	10.80	7.42	6.56	8.27	7.83	10.23	5.21	5.87	6.65	5.20	7.40
II	Alfalfa[2]	12.21	12.07	8.28	4.71	7.84	10.12	9.62	9.43	10.00	8.86	9.31
	Timothy[2]	9.10	6.90	4.92	4.77	8.32	6.33	6.07	8.01	7.09	7.76	7.26
	Western rye[2]	8.60	8.43	6.28	5.52	8.84	10.61	6.61	8.40	9.53	9.37	8.89
III	Alfalfa					8.08	8.28	6.27	7.69	7.52	9.27	8.44
	Timothy					6.40	8.26	6.04	7.82	7.38	9.31	7.09
	Western rye					8.41	8.44	5.31	6.78	4.36	6.86	6.90
B-5	Fallow	11.23	10.71	8.66	7.72	11.48	15.22	8.53	6.54	6.07	6.19	9.23
B-5	Fallow (variability)	10.33	10.43	8.44	8.83			7.84			7.62	8.91
	Fungi, thousands per gm.											
I	Wheat[1] following alfalfa	24	31	16	17	32	29	17	20	19	25	23
	Wheat[1] following timothy	36	24	18	17	30	27	17	20	18	24	23
	Wheat[1] following western rye	44	17	19	17	27	26	21	16	24	17	23
II	Alfalfa[2]	114	44	39	52	53	58	33	53	64	39	46
	Timothy[2]	52	68	35	22	28	22	31	65	62	82	41
	Western rye[2]	55	47	25	23	34	13	46	62			50
III	Alfalfa					69	95	54	79	67	85	70
	Timothy					47	70	32	45	38	44	45
	Western rye					33	79	24	30	30	25	37
B-5	Fallow	35	13	33	43	37	58	39	39	39	43	38
B-5	Fallow (variability)	37	15	39	39			35			44	35

[1] Second crop of wheat after sod.   [2] Broken in July.

TABLE XXI

SEASONAL FLUCTUATIONS IN MOISTURE, AND NUMBERS OF MICRO-ORGANISMS IN UPPER 6⅔ IN. OF SOIL, UNDER ALFALFA, GRASSES, AND FALLOW, IN 1931

Sub-block	Crop	May 11	May 25	June 11	June 24	July 7	July 21	Aug. 11	Aug. 26	Sept. 10	Sept. 22	Average
	Moisture, %											
II	Wheat[1] after alfalfa	32.1	31.2	28.1	42.5	38.8	17.7	41.1	31.9	31.3	34.0	32.9
	Wheat[1] after brome	34.0	31.3	30.3	40.4	37.8	20.2	41.6	32.9	36.2	38.0	34.3
	Wheat[1] after timothy	31.9	31.3	30.0	39.3	34.6	19.6	39.2	29.5	32.0	34.5	32.3
	Wheat[1] after western rye	31.8	30.8	29.4	40.5	39.4	20.5	42.7	31.6	35.6	38.0	34.0
III	Alfalfa	29.3	20.9	23.9	40.6	38.6	18.6	42.7	32.5	27.5	30.7	30.5
	Brome	29.2	21.5	22.9	43.4	36.7	22.8	43.7	32.6	33.1	39.8	32.6
	Timothy	26.5	19.0	21.7	39.7	39.5	21.7	43.3	30.0	32.7	37.0	31.1
	Western rye	29.1	24.3	24.4	42.6	36.8	24.5	41.0	32.7	28.8	36.1	32.0
A-1	Fallow	30.2	26.6	26.6	40.9	37.1	29.5	39.8	34.3	35.0	38.4	33.8
A-1	Fallow (variability)		28.6		40.4	39.4	29.2	40.0	31.4	34.3	37.6	35.1
	Bacteria, millions per gm.											
II	Wheat[1] after alfalfa	36.14	11.60	9.49	14.41	15.87	4.35	19.21	8.85	6.42	5.54	13.19
	Wheat[1] after brome	28.44	10.57	7.43	14.30	17.20	4.75	16.10	4.61	5.67	10.28	11.93
	Wheat[1] after timothy	26.53	7.81	8.17	11.21	12.50	3.45	10.01	2.28	5.51	7.98	9.54
	Wheat[1] after western rye	24.55	8.08	8.16	12.20	13.46	4.96	12.45	7.68	6.38	10.83	10.87
III	Alfalfa	18.39	6.26	6.89	10.31	15.75	4.68	17.43	8.45	8.60	6.35	10.31
	Brome	18.68	8.95	7.54	8.46	13.60	4.89	15.10	8.55	8.64	12.66	10.71
	Timothy	12.76	6.98	7.54	11.24	12.84	4.42	17.90	7.95	10.53	9.25	10.14
	Western rye	15.58	7.70	6.39	7.49	10.26	4.08	16.96	8.14	6.25	9.05	9.19
A-1	Fallow	10.24	6.58	3.38	10.90	10.16	4.80	8.86	6.17	4.48	7.57	7.23
A-1	Fallow (variability)		8.55		11.83	10.43	4.52	8.80	5.04	5.80	6.85	7.13
	Fungi, thousands per gm.											
II	Wheat[1] after alfalfa	10	23	21	21	25	31	53	44	20	18	26
	Wheat[1] after brome	87	132	165	281	257	91	237	139	139	96	162
	Wheat[1] after timothy	19	21	18	36	32	21	37	32	21	22	26
	Wheat[1] after western rye	24	27	38	64	60	34	55	70	43	96	46
III	Alfalfa	47	27	33	49	74	34	65	64	52	36	48
	Brome	14	12	7	9	18	11	29	16	14	16	14
	Timothy	13	17	15	14	23	13	33	29	21	21	20
	Western rye	15	12	10	19	19	16	25	39	21	24	20
A-1	Fallow	16	15	9	30	27	19	32	38	23	25	23
A-1	Fallow (variability)		22		36	31	16	31	35	25	27	28

[1] First crop of wheat after sod.

TABLE XXII

SEASONAL FLUCTUATIONS IN MOISTURE, AND NUMBERS OF MICRO-ORGANISMS IN UPPER 6⅜ IN. OF SOIL, UNDER ALFALFA, GRASSES, AND FALLOW, IN 1932

Sub-block	Crop	May 19	June 2	June 19	July 2	July 19	Aug. 2	Aug. 19	Sept. 2	Sept. 19	Av.
					Moisture, %						
II	Wheat[1] after alfalfa	31.8	28.4	26.4	27.0	20.6	16.5	13.4	13.3	26.2	22.6
	Wheat[1] after brome	31.0	27.8	26.6	26.4	21.5	16.6	12.2	12.7	27.6	22.5
	Wheat[1] after timothy	29.5	27.5	26.3	25.2	19.1	16.4	13.2	13.7	25.9	21.9
	Wheat[1] after western rye	29.0	22.9	27.5	23.9	22.2	13.5	13.6	12.3	28.6	21.5
III	Alfalfa[2]	24.6	22.9	22.3	19.0	20.2	16.2	12.8	14.7	27.5	20.0
	Brome[2]	29.0	27.0	25.9	26.2	25.1	21.0	14.4	16.5	30.2	23.9
	Timothy[2]	24.0	25.0	20.4	25.2	21.4	17.6	13.9	14.8	30.3	21.4
	Western rye[2][3]	25.5	23.8	21.7	27.7	24.2	20.0	15.7	16.8	27.5	22.5
	Fallow	29.0	28.0	28.9	33.1	43.1	37.3	29.4	32.5	36.7	33.1
	Fallow	28.9	28.0	29.0	33.3	42.3	39.9	31.6	32.5	37.6	33.7
					Bacteria, millions per gm.						
II	Wheat[1] after alfalfa	7.6	9.3	6.7	7.0	6.1	5.5	5.8	6.3	8.0	6.9
	Wheat[1] after brome	11.8	8.7	7.1	3.7	5.7	5.3	5.7	3.1	7.0	7.4
	Wheat[1] after timothy	20.4	9.9	7.5	6.1	3.9	5.0	4.8	6.0	7.3	7.9
	Wheat[1] after western rye	10.0	8.0	7.0	6.1	5.7	4.6	4.5	4.8	8.7	6.6
III	Alfalfa[2]	14.8	10.9	13.0	6.1	5.0	3.7	5.5	4.5	9.7	8.1
	Brome[2]	14.1	12.4	7.8	8.3	4.3	3.8	5.1	5.1	9.7	7.8
	Timothy[2]	8.8	12.7	4.3	6.0	2.6	3.2	5.0	5.9	9.8	6.5
	Western rye[2][3]	10.3	9.2	5.3	6.0	3.9	4.2	4.3	4.4	10.0	6.4
	Fallow (variability)	13.5	12.3	3.2	7.7	7.0	5.1	6.5	7.9	9.7	8.1
	Fallow (variability)	13.8	11.8	8.1	7.6	7.3	6.9	6.8	7.9	10.0	8.9
					Fungi, thousands per gm.						
II	Wheat[1] after alfalfa	9	20	18	17	15	8	14	10	15	14
	Wheat[1] after brome	19	62	52	24	55	5	22	23	40	33
	Wheat[1] after timothy	10	32	21	17	120	6	23	37	26	32
	Wheat[1] after western rye	10	32	24	31	20	3	40	9	35	23
III	Alfalfa[2]	21	68	35	24	43	1	32	26	15	29
	Brome[2]	14	38	11	12	12	4	58	29	30	23
	Timothy[2]	13	49	15	11	10	6	16	23	42	20
	Western rye[2][3]	11	38	8	16	18	13	12		47	20
	Fallow (variability)	7	54	20	26	17	17	29	38	22	25
	Fallow (variability)	6	56	19	30	25	24	37	41	34	30

[1] Second crop of wheat after sod.   [2] Broken in July.   [3] Killed by "take-all".

TABLE XXIII

SEASONAL FLUCTUATIONS IN MOISTURE, AND NUMBERS OF MICRO-ORGANISMS IN UPPER 6$\frac{3}{4}$ IN.
OF SOIL, UNDER ALFALFA, GRASSES, AND FALLOW, IN 1933

Sub-block	Crop	May 3	May 19	June 2	June 17	Average
		Moisture, %				
III	Wheat[1] after alfalfa	33.7	51.0[2]	29.6	26.9	35.3
	Wheat[1] after brome	34.6	48.9[2]	32.2	27.9	35.9
	Wheat[1] after timothy	33.7	47.5[2]	32.3	25.1	34.6
	Fallow	35.3	42.3[2]	31.7	36.1	36.3
	Fallow (Variability)	30.9	42.3[2]	34.5	33.5	35.3
		Bacteria, millions per gm.				
III	Wheat[1] after alfalfa	16.2	17.5	10.6	7.2	12.9
	Wheat[1] after brome	15.0	18.3	7.5	9.8	12.6
	Wheat[1] after timothy	12.9	23.5	8.8	9.1	13.6
	Fallow	12.5	14.3	10.8	10.7	12.1
	Fallow (Variability)	12.4	14.4	9.1	11.0	11.7
		Fungi, thousands per gm.				
III	Wheat[1] after alfalfa	16	19	30	20	21
	Wheat[1] after brome	64	42	61	60	57
	Wheat[1] after timothy	122	59	53	51	71
	Fallow	49	46	58	56 ·	52
	Fallow (Variability)	38	69	64	56	57

[1] *First crop of wheat after sod.*
[2] *Shortly after a shower of rain.*

The moisture content of the surface soil in the different plots fluctuated greatly during the seasons, especially in 1930 and 1931. However, the differences between the different plots or crops in this respect were comparatively small. The moisture varied from about 10 to 37% in 1930, from 17 to 43% in 1931, and from 12 to 32% in 1932.

The numbers of fungi did not fluctuate very greatly except under the first crop of wheat following brome grass in 1931, when the numbers were very much greater than those under the first crop of wheat following timothy, western rye grass, and alfalfa. The numbers varied from about 80 to 280 thousands per gram following brome grass, but only from about 10 to 100 thousands per gram following timothy, western rye grass, and alfalfa. The high counts following brome grass were accounted for mainly by the development of relatively large numbers of small Penicillium colonies on the acid agar plates inoculated with this soil (Fig. 5). This was the first season in which fungi counts were made following the plowing down of brome grass.

There is definite evidence of correlation between soil moisture and numbers of fungi under the first crop of wheat following brome grass in 1931.

Following the plowing down of the Sub-block II sod plots in 1930, it was observed that large Mucor colonies developed rapidly on the plates inoculated with soil from the alfalfa plots, and possibly suppressed the growth of other, slower-growing fungi (Figs. 3 and 4). A similar effect was observed the following year under the first crop of wheat following alfalfa, and in 1932 under the second crop of wheat. The fungi counts (mainly Mucor) were rather small under the first and second crops of wheat following alfalfa, the seasonal average being only 14 thousand per gram of soil under the second.

The numbers of bacteria (including actinomycetes) did not fluctuate very greatly except under the first crop of wheat in 1931, as in the case of the fungi. The numbers varied as a rule from about 6 to 10 millions per gram in 1930, and from about 4 to 10 millions per gram in 1932. However, in the moister season of 1931 the numbers fluctuated a good deal more in all plots. They were extremely high in the spring and afterwards fluctuated between about 4 and 18 millions per gram. The differences between the effects of the different preceding crops were not very significant, but in 1931 especially, the fluctuations in bacterial numbers were obviously correlated with fluctuations in the moisture content of the soil.

The numbers of micro-organisms in the alfalfa, timothy, and western rye grass plots of Sub-block III were determined in 1929, 1930, 1931 and 1932, and in the brome grass plots in 1931 and 1932. In the early part of the 1933 season, when the plots were producing the first crop of wheat, counts were made in the alfalfa, brome, and timothy plots, but not in the western rye grass plots, because these had previously become badly infested with root rot.

Counts were also made of the micro-organisms in the supplementary fallow plots used for the nitrification measurements from 1929 to 1933, in order to compare the numbers present in the cropped plots of the main experiment with the numbers present under conditions known to favour increase in moisture and accumulation of nitrates. During the four seasons, 1930 to 1933, two sets of composite samples were taken from the fallow plots as a rule, in order to study the variability of the composite samples.

The moisture content of the surface soil in the different plots fluctuated considerably, but there was less fluctuation in 1932 than in the other four years. The differences between the cropped plots were small as a rule, but, in every case except the relatively wet season of 1931, the moisture content of the fallow plot soil was higher in the latter part of each season than that of the cropped plots.

The numbers of fungi fluctuated considerably during the five seasons, 1929 to 1933, varying commonly from about 10 to 80 thousands per gram of soil. During three out of four of the hay crop years, the fungi counts were generally higher in the alfalfa plots than in any of the grass plots; this applies to 1929, 1930, and especially to 1931, but not to 1932. Under the first crop

of wheat, in 1933, it was observed that, as in the cases of Sub-blocks I and II, Mucor colonies predominated on the plates inoculated with soil from plots in which alfalfa had been turned under (Fig. 3). The counts were relatively low in these plots, thus agreeing with the results obtained in Sub-block II.

The number of bacteria (including actinomycetes) fluctuated considerably during the five seasons, 1929 to 1933, in which counts were made. During the first four of these, the Sub-block III plots were under alfalfa and grass. The numbers varied from about 4 to 12 millions per gram of soil in 1929 and 1930. In the moister season of 1931 the numbers varied from about 4 to 18 millions per gram, and from about 2 to 14 millions in 1932. Under the first crop of wheat, in 1933, counts were limited to May and June and varied from about 6 to 18 millions per gram of soil. The differences between the effects of the different crops (alfalfa, brome, timothy, and western rye grass) were not very significant. Neither was there a significant difference between the effects of the different crops and fallow. However, the seasonal fluctuations in bacterial numbers were obviously correlated with fluctuations in the moisture content of the soil in all five seasons.

The numbers of actinomycetes did not fluctuate very much during the one season, 1929, when counts were made in the alfalfa and grass plots of Sub-block III. They varied usually between one-half million and two millions per gram of soil. The counts obtained in a nearby fallow plot in 1929 did not fluctuate very much either.

During the season of 1930 the numbers of amoebae were determined by the dilution plate count method, in the alfalfa and timothy plots of Sub-block III, and in the fallow plots. Throughout this season amoebae were nearly always found in the $\frac{1}{1000}$ dilution plates but not in the $\frac{1}{10,000}$ dilution plates.

Distribution of Bacteria and Fungi in the Soil Profile Layers

In the main experiment, soil samples for microbial counts were taken to a depth of $6\frac{2}{3}$ in., or to the plow depth approximately, as it is generally believed that most of the microbial activity takes place in this upper layer of soil. However, a supplementary experiment was carried out during the season of 1932 in order to obtain a better idea of the distribution of bacteria and fungi in the Edmonton soil profile layers. The samples were taken from nearby cultivated and old sod plots. Large holes were dug in these plots to a depth of 36 in., and successive samples were obtained at later dates by digging back into the wall of soil, and thus taking samples well back in the unexposed soil each time. The plots were sampled five times during the season, and the counts obtained are given in Table XXIV.

Although in the cultivated plot the surface layers generally contained most bacteria, the deeper samples gave fairly high counts in both the cultivated and sod plots. The fungi counts in the cultivated plot were generally highest in the surface layer, but in the sod plot the deeper samples gave fairly high counts.

TABLE XXIV

SEASONAL FLUCTUATIONS IN NUMBERS OF MICRO-ORGANISMS AT VARIOUS DEPTHS IN CULTIVATED AND GRASS SOD EDMONTON SOIL, IN 1932

Depth, in.	Horizon	May 30	June 30	July 30	Aug. 30	Sept. 30	Average
		Fungi in cultivated soil, thousands per gm.					
0 – 3	A₁	35	8	10	15	22	18
3 – 6	A₁	30	6	6	4	5	10
6 – 12	A₁	3	2	3	3	6	3
14 – 22	A₂	2	2	1	5	5	3
22 – 29	B₁	1	6	0	3	5	3
29 – 36	B₂	0	0	1	2	5	2
		Fungi in grass sod soil, thousands per gm.					
0 – 3	A₁	19	15	38	44	7	25
3 – 6	A₁	12	7	13	10	4	9
6 – 12	A₁	13	4	5	5	4	6
14 – 22	A₂	6	19	7	19	21	14
22 – 29	B₁	4	18	17	12	25	15
29 – 36	B₂	9	18	37	21	14	20
		Bacteria in cultivated soil, millions per gm.					
0 – 3	A₁	13.0	6.5	4.2	6.7	11.3	8.3
3 – 6	A₁	10.4	4.8	4.1	5.8	2.8	5.6
6 – 12	A₁	3.2	1.1	1.5	1.9	2.5	2.0
14 – 22	A₂	5.2	0.7	2.2	2.9	3.7	2.9
22 – 29	B₁	3.9	0.9	2.3	2.6	4.1	2.8
29 – 36	B₂	2.6	0.5	4.6	2.0	4.4	2.8
		Bacteria in grass sod soil, millions per gm.					
0 – 3	A₁	9.0	0.9	2.9	6.3	3.5	4.5
3 – 6	A₁	5.2	1.7	2.5	2.4	2.0	2.8
6 – 12	A₁	4.8	0.8	2.1	2.8	2.2	2.5
14 – 22	A₂	8.6	4.1	2.9	6.7	3.3	5.1
22 – 29	B₁	5.4	1.3	1.7	2.6	3.8	3.0
29 – 36	B₂	5.8	1.5	0.5	5.7	4.4	3.6

TOTAL NITROGEN

Table XXV shows the total nitrogen content of the alfalfa, brome, timothy, and western rye grass plot soils and of the wheat plots in the three sub-blocks. The nitrogen determinations were made once a year, on samples of surface, subsurface, and subsoil taken for the nitrate determinations. The surface soil in every case contained most nitrogen and the subsoil least. Although the samples analyzed were composites, there was considerable variation from year to year which cannot reasonably be attributed to actual changes in soil composition, but must arise from random sampling variations. The maximum variation in the nitrogen content of the composite samples of surface soil

TABLE XXV

TOTAL NITROGEN CONTENT OF EDMONTON PLOT SOILS, DETERMINED YEARLY

Sub-block	Replicates	Crop	Depth, in.	June 15, 1927	May 17, 1928	May 14, 1929	May 12, 1930	May 13, 1931	May 17, 1932	May 7, 1934
I	1–7–12–14	Timothy	0–6⅔	0.63	0.58	0.54	0.58	0.52	0.52	0.58
			6⅔–20	0.26	0.22	0.33	0.29	0.20	0.20	0.28
			20–40	0.10	0.09	0.08	0.10	0.09	0.08	0.09
I	2–8–11–13	Alfalfa	0–6⅔	0.54	0.53	0.47	0.56	0.54	0.53	0.52
			6⅔–20	0.25	0.21	0.26	0.31	0.24	0.24	0.31
			20–40	0.10	0.10	0.09	0.10	0.08	0.08	0.07
I	3–6– 9–16	Brome	0–6⅔	0.57	0.58	0.55	0.58	0.52	0.51	0.54
			6⅔–20	0.23	0.22	0.22	0.31	0.24	0.24	0.32
			20–40	0.10	0.12	0.08	0.12	0.08	0.08	0.06
I	4–5–10–15	W. rye	0–6⅔	0.58	0.56	0.53	0.59	0.46	0.44	0.58
			6⅔–20	0.23	0.30	0.33	0.31	0.32	0.26	0.30
			20–40	0.10	0.10	0.10	0.10	0.11	0.11	0.08
II	1–6–12–15	Alfalfa	0–6⅔	0.57	0.59	0.64	0.62	0.60	0.59	0.64
			6⅔–20	0.23	0.24	0.31	0.22	0.26	0.25	0.31
			20–40	0.10	0.10	0.13	0.11	0.09	0.08	0.08
II	2–7–9–16	Brome	0–6⅔	0.65	0.53	0.61	0.61	0.61	0.59	0.62
			6⅔–20	0.23	0.23	0.27	0.26	0.22	0.23	0.37
			20–40	0.10	0.10	0.16	0.10	0.13	0.10	0.08
II	3–8–10–13	Timothy	0–6⅔	0.62	0.56	0.51	0.60	0.58	0.56	0.59
			6⅔–20	0.22	0.24	0.22	0.29	0.27	0.26	0.28
			20–40	0.10	0.09	0.20	0.10	0.08	0.07	0.08
II	4–5–11–14	W. rye	0–6⅔	0.62	0.56	0.58	0.59	0.60	0.59	0.63
			6⅔–20	0.29	0.23	0.32	0.28	0.30	0.26	0.31
			20–40	0.11	0.09	0.22	0.11	0.09	0.08	0.08
III	1–8–10–15	Timothy	0–6⅔	0.70	0.65	0.65	0.66	0.66	0.65	0.69
			6⅔–20	0.29	0.28	0.41	0.30	0.31	0.26	0.36
			20–40	0.11	0.10	0.29	0.12	—	0.08	0.08
III	2–5–11–16	Alfalfa	0–6⅔	0.70	0.66	0.72	0.66	0.70	0.68	0.72
			6⅔–20	0.32	0.22	0.32	0.28	0.27	0.28	0.37
			20–40	0.12	0.09	0.18	0.10	0.08	0.08	0.08
III	3–6–12–13	Brome	0–6⅔	0.70	0.74	0.69	0.70	0.68	0.67	0.73
			6⅔–20	0.25	0.30	0.25	0.26	0.28	0.27	0.28
			20–40	0.11	0.10	0.20	0.10	0.08	0.08	0.09
III	4–7–9–14	W. rye	0–6⅔	0.69	0.69	0.69	0.66	0.64	0.63	
			6⅔–20	0.32	0.30	0.42	0.27	0.38	0.27	
			20–40	0.11	0.10	0.20	0.10	0.12	0.09	

throughout the seven yearly determinations was 0.12%. This happened in the samples from the brome grass plots of Sub-block II. The variations showed no definite trend downwards or upwards throughout this period of years.

Discussion

Soil microbiological activity was measured in this experiment in order to study some underlying causes of the comparative effects of alfalfa, brome,

timothy, and western rye grass on the yield and nitrogen content of succeeding wheat crops.

It was previously shown at Edmonton that relatively large amounts of nitrate are produced in Edmonton black soil after clover sod is plowed down (32, 33), and a number of investigators, including Lyon, Bizzell, and Wilson at Cornell (14), had shown that greater amounts of nitrate are produced in the soil following crops of clover and alfalfa, or after these crops have been plowed under, than following cereals and grasses. However, the effect of alfalfa on the yield and nitrogen content of succeeding wheat crops, as well as on nitrification had not been investigated previously at Edmonton.

Investigations by Albrecht (1) and others have shown that nitrification is depressed by timothy. It was therefore desirable to compare the effect on nitrification of timothy with that of brome and western rye grass, as well as alfalfa. It was also important to compare nitrification under wheat following these different grasses and alfalfa, in relation to the yield and nitrogen content of the wheat crop.

When the previously fallowed soil was seeded to alfalfa and grasses, the moisture and nitrate content of the soil were reduced, and generally remained at a relatively low level until the sods were plowed up. In previously reported experiments (17, 32, 33) it was found that whereas fallow land at Edmonton usually contained most soluble nitrate nitrogen, and soils supporting the ordinary grain crops contained intermediate amounts, the perennial crop soils, and particularly the grass crop soils, usually contained least. In the experiments reported in this paper the nitrates were reduced to a very low level or disappeared entirely in the grass and alfalfa plots in the drier seasons, but increased considerably in the moister seasons. The nitrate content of the alfalfa plot soils was generally greater than that of the grass plots, and the brome plots were generally lower in nitrates than the other grass plot soils.

The nitrate content of the grass and alfalfa plot soils was generally lower than that of the wheat plots, especially in the drier seasons. The high nitrate content of the fallow plots may be explained in large measure by the higher moisture content of the fallow plots and the lack of absorption by crops, but the explanation for the higher nitrate content of the wheat plot soil as compared to the perennial plot soil is not very clear. The moisture content of the grain plots was not appreciably greater than that of the perennial crop plots. The cultivation before seeding grain may have improved the physical condition of the soil and thus increased nitrification. However, some other explanation based upon crop characteristics may account for the difference.

When alfalfa plots were plowed, nitrification proceeded more rapidly, or nitrates accumulated to a greater extent, than in the plowed grass plots. The greater nitrate content of the soil under wheat following alfalfa was observed for a period of three or four years following the plowing of the sods in separate sub-blocks plowed up two years apart. The greater nitrate production can be explained most satisfactorily, probably, on the basis of the higher nitrogen content of alfalfa residues as compared to grass residues.

The greater nitrification following alfalfa has an important effect on the composition of crops, as shown by the fact that the protein content of wheat in this experiment was considerably higher following alfalfa than following the grasses for the period of three or four years (19). In other experiments on gray, wooded soils of Alberta, it has been shown that the application of ammonium salt fertilizers at seeding time has very little effect on the protein content of wheat, but that the plowing down of clovers produces a marked increase in the protein content of the succeeding wheat crop, probably because the nitrogen of the clover becomes available gradually during the growing season (35). It is interesting to find that this effect of legumes on the protein content of succeeding wheat crops is produced even in the relatively fertile Edmonton black soil.

Not only were the brome grass plots generally lower in nitrates than the other grass plots, but the wheat plots following brome were also generally lower in nitrates than following timothy or western rye grass. This lower nitrification was reflected in a smaller absorption of nitrogen by the wheat crop and a lower protein wheat following brome than following the other two grasses. The reason why the nitrogen of brome grass residues is less available than that of the other grasses is not definitely known. The brome grass residues may be decomposed with greater difficulty by soil micro-organisms, although the average carbon to nitrogen ratio of the residues is no wider than that of timothy, in these experiments, and but slightly wider than that of western rye grass. According to Russell (22, p. 313) and others, the nitrogen of soil organic matter can appear as nitrate only if it exceeds a certain critical amount relative to carbon; and when the proportion of carbon is greater, the nitrogen remains as complex protein. This critical ratio is usually 12, or less, of carbon to one of nitrogen, and until the excess of carbon is lost by decomposition, nitrate will not appear in the soil, or will be absorbed as rapidly as it is produced. Collison and Conn (3), Heukelekian, Waksman and Skinner (10, 27) and others have shown that cellulose is decomposed in the soil to a large extent by fungi, which require a source of available nitrogen.

The absorption of nitrogen by the wheat crop indicates the rate of soil nitrification, as stated in the first paper of this series (19). However, a better indication is given by both absorption of nitrogen and accumulation of nitrate in the soil. In these experiments the greatest absorption occurred under wheat following alfalfa, where nitrate accumulated to the greatest degree, and the least under wheat following brome, where nitrate accumulated to the smallest degree.

The tendency of the nitrate content of the soil under wheat to drop in mid-summer may be explained satisfactorily by the fact that this corresponds with the period of maximum absorption of nitrates by the growing crop. The effect of the growing crop is further shown by a comparison with the fallow plots. The nitrate content of the fallow soil fluctuates, but does not show this tendency to drop in mid-summer, and the fallow plots were relatively high in both nitrate and moisture at the end of each season.

Several investigators, including King and Whitson (12) and Russell (21), showed that the period of loss of nitrate in the soil corresponded with the period of rapid growth of crops. King and Whitson (12), Jensen (11), Russell (21), and Whiting and Schoonover (29) found that the most active period of production and accumulation of nitrates was the late spring and early summer, and that there was an increase in nitrification in the early autumn, following a period of low nitrification in the late summer. However, neither early summer nor fall increases in nitrification were obtained consistently in the fallow plots at Edmonton, and nitrate accumulation was not interfered with by crop growth on these plots. These results tend to support the contention of Lemmermann and Wichers (13), who believe that physical factors affect the nitrification process, and contend that there is not sufficient evidence to show a direct periodic influence of the time of the year on the life activities of the organisms.

The different crops of this experiment apparently utilized about the same amounts of soil moisture, as the differences between the moisture content of the different plots were not great. However, in Sub-block III the oldest stand of alfalfa in the experiment was plowed up in July 1932, and the mositure content of the soil was lower throughout that season in these alfalfa plots than in any of the grass plots. Duly (6) found that old stands of alfalfa reduced to an appreciable extent the moisture at the lower depths.

Broadly speaking, an increase in moisture is usually accompanied by an increase in nitrate accumulation, under a given crop, but there is no very close correlation between fluctuations in moisture and nitrate nitrogen. This lack of correlation may be explained in part by the fact that the moisture and nitrate determinations were made only once a month. Furthermore, it should be kept in mind that in all cases except the summerfallow, crops were growing on the plots and utilizing both nitrates and moisture. However, it will be observed that the general level of nitrates under alfalfa and grasses in Sub-blocks II and III dropped from season to season between 1927 and 1929, inclusive, as the moisture content of the soil decreased. Also, on comparing the dry seasons of 1929 and 1930 with the moister soil conditions of 1931 and the early part of 1932, one notices that the nitrate content of the continuous alfalfa and grass plots of Sub-block III was higher in 1931 and 1932 (Fig. 1), showing that an increase in moisture stimulates nitrification.

The distribution of nitrate nitrogen and moisture in the different depths is shown in Table XVII, in the form of seasonal averages (and averages of several seasons) for enough of the treatments and years to give a good idea of its nature. It will be observed that the average distribution of nitrate is rather similar whether the plots are supporting wheat (Sub-block I), alfalfa (Sub-block III), timothy (Sub-block III), or fallow, in spite of the large differences in the total amount present. The surface often contains about twice as high a concentration as the subsurface, and the subsurface about twice the subsoil. Nitrate is readily soluble and moves downward in the soil to a considerable extent by diffusion and movement of soil moisture. Occasion-

ally greater concentrations were found in the lower depths, and this may be accounted for by leaching or diffusion, and utilization near the surface by plants or micro-organisms. It is reasonable to believe that most of the nitrification occurs in the surface and that the nitrate in the subsoil has been brought down by diffusion or leaching from the upper layers.

The numbers of fungi and bacteria, as determined by the plate count method, did not fluctuate very much in certain plots and seasons, but fluctuated greatly in others. As in previous experiments (17), the fluctuations in nitrate nitrogen did not correspond closely to fluctuations in numbers of fungi, bacteria, or actinomycetes. The fluctuations are undoubtedly affected by food supply and other factors. Moisture is probably one of the most important of these factors, as there is evidence of a correlation between soil moisture and numbers of micro-organisms, especially in the case of bacteria.

The work of Cutler, Crump, and Sandon (5), and of Thornton and Gray (25), showed that the bacterial numbers vary considerably from one day to the next, and even from hour to hour. Superimposed on these fluctuations are the great seasonal changes in numbers observed by Russell and Appleyard at Rothamsted (23), and by other workers elsewhere. One object of the experiments reported in this paper was to measure these larger seasonal changes.

The importance of bacteria in the decomposition of plant residues has been generally recognized for a longer period of time than the importance of other soil micro-organisms. Of late years experimenters such as Waksman, Skinner, and Heukelekian (10, 27) have come to the conclusion that a large proportion of the decomposition and synthesis of organic matter within the soil is brought about by soil fungi. Increasing attention has been given lately to the competition between different groups of soil micro-organisms, and between higher plants and soil micro-organisms, for nutrients present in the soil.

Hiltner, many years ago, and others who have more recently investigated the influence of higher plants on micro-organisms, have shown that micro-organisms tend to accumulate close to the roots of plants. It has been demonstrated by Conn and others that the numbers of bacteria determined by the plate count method represent only a small fraction of the total number present in the soil (2).

Dr. F. J. Greaney, Professor N. James, Dr. J. E. Machacek, and Dr. W. L. Gordon found at Winnipeg, Manitoba, that the total bacterial counts (including actinomycetes) of replicate plates had an abnormal variance, deviating from what was expected according to the laws of probability. They found, however, that the distribution of the total number of fungi in replicate plates agreed well with the theoretical*. We therefore investigated the variability of replicate plate counts from given composite samples of soil**. The differences in counts between parallel plates should arise solely from random sampling variations in the number of micro-organisms withdrawn

from suspensions in each aliquot, and should therefore occur in accordance with Poisson's law, provided that all the organisms (or a constant proportion of them) deposited on the plates grow or produce colonies, and provided there are no discrepancies arising from deficiencies in manipulation, incubation, etc.   In order to determine whether the actual counts conformed to the theoretical requirements, it was necessary to calculate the index of dispersion appropriate to the Poisson distribution.   When this was done it was found that in the counts of bacteria (including actinomycetes) the probability of chance deviations of the magnitude obtained was remote.   In counts of fungi the agreement was better, but still unsatisfactory on the whole, although the replicate plate counts of some plots throughout certain seasons were within the limits of probable chance deviation.   It is quite likely that more of the replicate plate counts of fungi would have fallen within the limits of probable chance deviation if the medium had not contained protein, which tends to encourage the development of spreading proteolytic fungi.   Czapek's protein-free medium (8) was used in the experiments previously referred to, at Winnipeg.   The closer approximation of the fungal counts to the theoretical requirements, in spite of the known defects of the plate count method for fungi, may help to explain why the fungi counts seem to be of greater significance in these experiments than those for bacteria.

Fortunately, no abnormal variations were encountered in the parallel determinations of moisture, nitrate, and phosphate; for a given sample of soil there was close agreement between duplicates.

The abnormal variance in replicate plate counts from the same sample of soil makes it difficult to establish significant differences in counts between different soil samples.   The actual variation between different samples has been calculated from the results of counts of duplicate composite samples from the fallow plots in 1930 to 1933, shown at the bottom of each section in Tables XX to XXIII.   For bacteria (including actinomycetes) the standard error within dates varied from zero to a maximum of 0.7 millions per gram of soil (with the exception of one date in 1932) during all four seasons.   Thus, to be statistically significant when the difference between duplicate counts was greatest, within or between dates, the plus or minus difference from the mean of the duplicate counts would require to be 1.4 millions per gram.

For the counts of fungi, the standard error within dates varied from 0.3 to 2.1 thousands per gram of soil in 1930, from 0.3 to 2.5 thousands in 1931, from 0.3 to 4.2 thousands in 1932, and (with one exception) from zero to 5.5 thousands in 1933.   Here, statistically significant variations from the mean of duplicates, within or between dates, would need to be 4 thousand per gram in 1930, 5 thousand in 1931, 8.5 thousand in 1932, and 11 thousand in 1933.   Although the standard error of microbial counts of plots other than fallow cannot be calculated, it may be assumed, not unreasonably, that the sampling errors of the other plots are similar to those of the fallow plots.

It was rather disappointing to find that the differences between numbers of bacteria in the alfalfa, brome, timothy, western rye grass, and fallow plots

were not very significant.   In previously reported experiments at Edmonton (17) covering a period of only two seasons, the seasonal average number of bacteria was greatest in fallow land and least in grass land, corresponding to the highest and lowest average moisture content of the soil.   However, in the experiments reported in the present paper this correlation between seasonal average number of bacteria and seasonal average moisture content of soil often did not exist, in spite of the correlation between fluctuations of soil moisture and numbers of bacteria.

A spring maximum of bacterial numbers was observed in about half of the seasonal curves.   Spring maxima were observed in the earlier experiments at Edmonton (17), and both spring and fall maxima have been observed by other workers in more humid regions.

There is evidence of a correlation between soil moisture fluctuations and fungal count fluctuations under the first crop of wheat following brome grass in 1931, but otherwise there is less evidence of correlation between fungal counts and moisture fluctuations than there is between bacterial counts and moisture.   As in earlier experiments at Edmonton (17), some evidence of correlation between fluctuations in numbers of fungi and bacteria was observed, but these correlations were not obtained consistently.

A fungus colony developing on a plate medium may represent a small bit of mycelium or a mass of mycelia, a single dormant spore or a mass of spores, or a mixture of mycelia and spores, and consequently the plate count method has often been considered of doubtful value for determining numbers of fungi present in a soil.   But in spite of the known defects of this method the results seem to be of greater significance, in these experiments, than those for bacteria. Some interesting relationships between the different crops and crop residues and the numbers and kinds of fungi developing on the plates were observed.

The numbers of fungi were generally higher in the alfalfa plots than in the brome, timothy, or western rye grass plots, but the differences between these grass plots were apparently insignificant.

Under the first crop of wheat it was repeatedly observed that large Mucor colonies predominated in the alfalfa plot soil plates and the counts were relatively low (Fig. 3).   In a soil treated with alfalfa roots, according to Martin (15) the predominant types of mould were Mucor, Rhizopus and Alternaria.   The low counts do not necessarily indicate a small number of fungi or absence of other forms, because it was necessary to make the counts after only two days of incubation, on account of the rapid development of the Mucor colonies.   If counting was delayed the plates became so overgrown by the fungus that the separate colonies could not be distinguished (Fig. 4). Probably other, slower growing types of fungi were present in the soil, but on account of the rapid growth of Mucor on the plates, no opportunity was given them for development.   This opinion is confirmed by the fact that during the later part of the season, when the Mucor colonies did not spread with great rapidity on the plates, Penicillium colonies appeared on the third day of incubation, as illustrated in Fig. 4.   The great development of Mucor

may be explained by the fact that Mucor is a fast-growing proteolytic fungus, and alfalfa residues are rich in protein. When incorporated with the soil, alfalfa apparently forms a suitable medium for this fungus.

Under the first crop of wheat in 1931, the brome grass plot soils gave by far the highest counts of fungi, and these appeared on the plates mainly as small Penicillium colonies (Fig. 5). These may have been stimulated in the soil
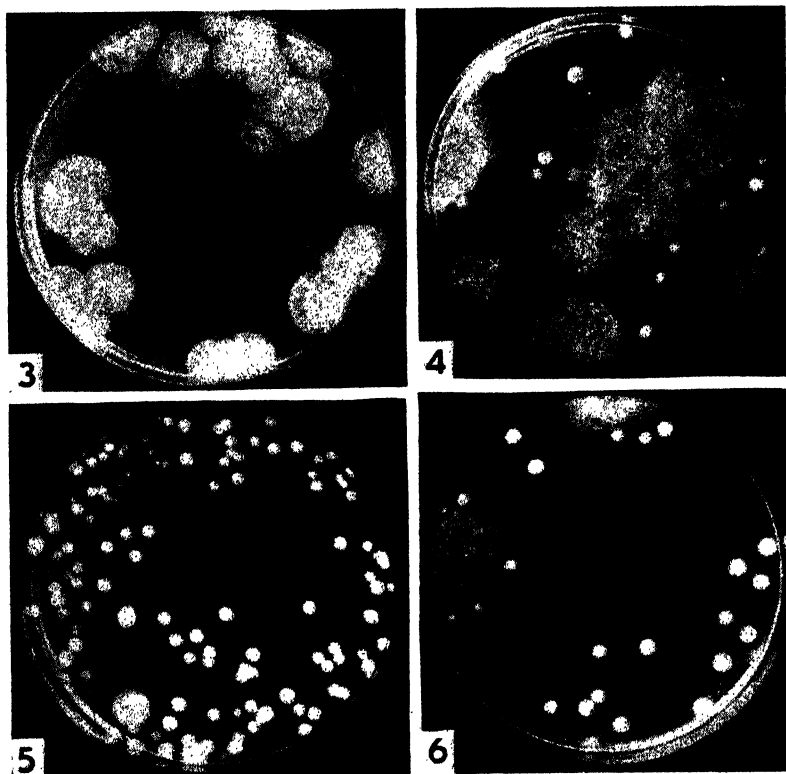
FIGS. 3 – 6. FIG. 3.—*Plated from soil after plowing down alfalfa. Note rapid development of Mucor after two days incubation. No other fungi are seen.* FIG. 4—*Plated from soil after plowing down alfalfa. Note the growth of Mucor and the development of other colonies (mainly Penicillia) after three days incubation.* FIG. 5—*Plated from soil after plowing down brome. Note great numbers of colonies, mainly Penicillia.* FIG. 6.—*Plated from soil after plowing down timothy. Note small numbers of colonies as compared with Fig. 5.*

by the incorporation of a large quantity of carbonaceous organic material of a character peculiar to brome grass. Apparently it is not a greater utilization or exhaustion of soil moisture by brome that is responsible for the poorer yield or quality of wheat crops following this grass, as the moisture contents of the soil of the different plots growing crops did not show any great differences. This would throw some doubt on the popular idea among farmers at the present time that brome causes poor growth of subsequent crops of wheat by drying out the land.

The numbers of actinomycetes, as determined by the plate count method, did not fluctuate very greatly during the one season in which they were determined, and the differences between the different plots were apparently insignificant. As in previous experiments (17), the actinomycetes were fairly numerous, but considerably less numerous than the bacteria. However, it is doubtful whether plate counting methods for actinomycetes are reliable, as a considerable number of bacterial colonies develop on the medium and often cannot be distinguished readily from actinomycetes colonies.

Apparently the numbers of amoebae present in Edmonton soil are relatively small compared, for example, to the numbers present in Rothamsted soils, where the interrelationship of soil protozoa and bacteria has been extensively investigated. At Rothamsted the number of amoebae present was usually more than 100,000 per gram of soil (24, p. 90) whereas at Edmonton the number was less than 10,000 per gram. The numbers of bacteria in the Edmonton soil, as determined by plate count methods, were usually much smaller than the numbers present in Rothamsted soil, and a few daily counts made in 1929 indicate that daily fluctuations are not nearly as marked in the Edmonton soil.

In the supplementary experiment on the distribution of bacteria and fungi in the Edmonton soil profile layers, it was found that the deeper layers gave surprisingly high counts of bacteria in both cultivated and sod plots, and that the deep samples from the sod plot gave surprisingly high counts of fungi, as shown in Table XXIV. However, the activity of the micro-organisms cannot be proportional to these counts, for there is relatively little activity in the subsoil.

The quantities of water-soluble phosphorus never exceeded about 14 parts per million in any of the plots throughout the one season in which this determination was made, and there were no pronounced fluctuations during the the growing season. The greatest quantities were found in the surface soil and the least in the lowest depth samples. The differences between different plots were not very significant. Most, if not all, of the water-soluble phosphorus is probably in organic combination, and may not be readily available to higher plants, but it has been shown by other investigators that plants can make use of some organic phosphorus compounds (28, 30).

A comparison of the seven successive yearly total nitrogen determination figures for the alfalfa, brome, timothy, and western rye grass plot soils, and wheat plot soils following these sods, shows that there was considerable variation from year to year. In no series of successive nitrogen determinations was the maximum variation less than 0.05% in the surface soil, and it was as great as 0.15% in one series. The variations cannot reasonably be attributed to actual changes in soil composition, and must arise mainly from random sampling variations. Each sample analyzed was a composite prepared from samples of four separate plots, which might account for a greater variation than would be obtained if the composites had been prepared from single plots. Nevertheless, these results emphasize the importance of sampling

error in any study of changes in the total nitrogen content of a soil produced by a given treatment. The changes in a period of seven years as measured in this experiment are obviously insignificant.

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RESEARCHES ON DROUGHT RESISTANCE IN SPRING WHEAT

I. A MACHINE FOR MEASURING THE RESISTANCE OF PLANTS TO ARTIFICIAL DROUGHT[1]

By Cyril B. Kenway[2] and H. B. Peto[3]

Abstract

The "chinook machine", designed and constructed by Dr. O. S. Aamodt, was reconstructed to provide economy of operation by the installation of a return air conductor, and uniform conditions for all plants exposed to treatment at one time, by means of a travelling belt to which the pots are fastened.

Since the publication of the results of the drought investigations conducted by Aamodt and his associates (2, 3), numerous experimental data on field and laboratory tests have been assembled. This paper is concerned only with a description of alterations and improvements made in the "chinook machine" originally described by Aamodt (1).

The original machine was characterized by two serious defects. In the first place, the warm air escaping from the end of the chamber into the greenhouse resulted in unduly high greenhouse temperature and excessive cost. The other defect was concerned with the impossibility of subjecting all the pots in one batch to similar conditions. Both temperature and air current velocity varied from one part of the chamber to another.

In order to avoid overheating the greenhouse and to reduce the cost of operation, a return air conductor was installed. The conductor was provided with horizontal partitions to reduce eddying, and with small adjustable intake and outlet openings for the purpose of ventilation. As a result of this change, the time required to bring the chamber up to a temperature of 110° F. was reduced from about two hours to 8 or 10 min., the consumption of electricity was greatly reduced, and the greenhouse temperature was only slightly raised during the tests.

Essentially uniform conditions for all pots in one batch were insured by the installation of a travelling belt to which containers for the pots were attached. The original floor was slightly lowered and covered with 24-gauge galvanized iron sheets to make a suitable base for the moving pots. At each end of the chamber an 8-in. sprocket was installed, the one next to the fan being fastened to the floor, while the other was connected directly to a gear reduction assembly. The latter was adapted from an automatic coal stoker, and had a gear reduction of 1800 to 1. The gear assembly was driven by a ¼ h.p. electric

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motor. An endless steel chain taken from the conveyor belt of a threshing machine was fitted to these sprockets. Galvanized cylindrical cans, supported by small furniture castors, were fastened to the chain by door springs (Fig. 2).
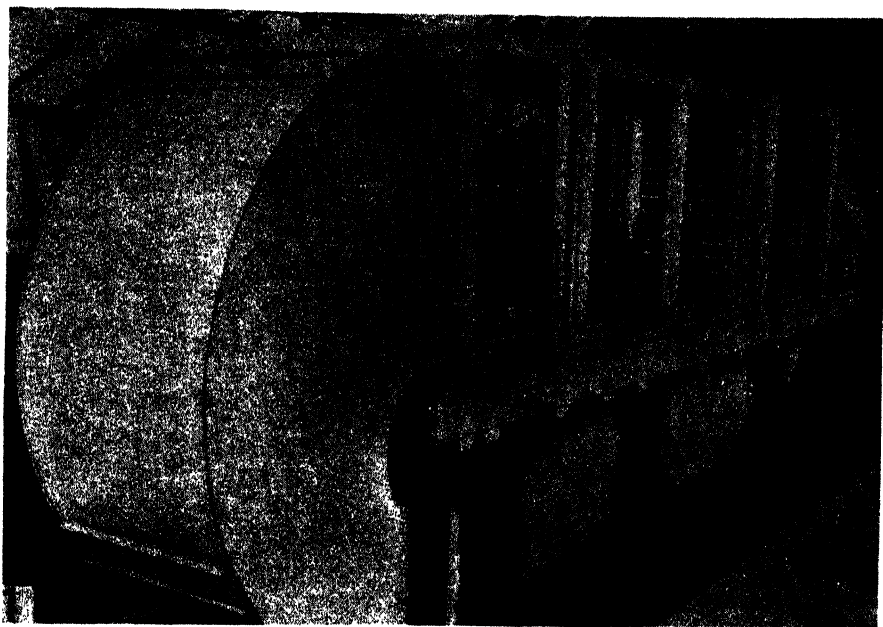
FIGS. 1 and 2. 1. *General view of chinook machine.* 2. *Interior view of chinook machine, illustrating pot containers and the method of their attachment to the endless chain.*

During treatment, the experimental material is in continuous motion, each pot making one complete revolution in six minutes. By this means, essentially uniform conditions for all material in any one batch are provided. The only drawback to this change is that the capacity of the machine is reduced from about 40 to 25 pots.

The alterations described above are illustrated in Figs. 1 and 2.

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A MUTATION FOR PATHOGENICITY IN PUCCINIA GRAMINIS TRITICI[1]

BY MARGARET NEWTON[2] AND THORVALDUR JOHNSON[3]

Abstract

A pathogenic change, explainable only on the assumption of mutation, has occurred in a uredial culture of race 52 of *Puccinia graminis Tritici*, which had previously remained constant in pathogenicity for nearly two years. The mutation appears to have taken place during a six-month period of storage of the urediospores in a refrigerator maintained at a temperature of about 8° C. When cultured in the greenhouse, at the end of this period, the rust appeared to be a mixture of race 52 and a hitherto undescribed physiologic race, with the latter predominating. The original culture was left in storage for a further period of four months, after which it gave rise to a pure culture of the new race without any indication of the presence of race 52. The new race has been assigned the number 178.

Although physiologic races of the various cereal rusts appear for the most part to remain constant in their pathogenic properties year after year, there have been reports, nevertheless, of sudden changes (mutations) in spore colour or pathogenicity. In *Puccinia graminis Tritici* Erikss. & Henn., mutation for uredial colour has been reported by Waterhouse (7) and by Newton and Johnson (3), while mutation for pathogenicity has been recorded by Stakman, Levine, and Cotter (6). In other cereal rusts, evidence for mutation has been presented by Johnston (2) and by Roberts (5) for *Puccinia triticina* Erikss.; by Gassner and Straib (1) for *Puccinia glumarum* (Schmidt) Erikss. & Henn.; and by d'Oliveira (4) for *Puccinia anomala* Rost.

In the greenhouse studies, the present writers have occasionally noticed apparently spontaneous pathogenic changes involving partial or entire displacement of one physiologic race by another. Owing to the fact that such changes have usually given rise to races already known and studied in the greenhouse at some previous time, it has never appeared safe to attribute them to mutation. Recently, however, a physiologic race has undergone a pathogenic change in which the original race was displaced by one hitherto undescribed. In this instance it does not appear possible to explain the change except by assuming that mutation occurred.

The pathogenic change occurred in a ·culture of race 52 derived from a selfing* of race 56 originally collected at Arnes, Manitoba, in 1934. In the selfing study, which was carried out in the fall of 1935, 21 uredial cultures, each arising from a single aecium, were identified. Culture No. 8 was identified as race 52 on January 8, 1936, and was placed in storage in a refrigerator kept at a low temperature (about 8° C.) on March 3. It was removed from the refrigerator in October of the same year and was cultured in the greenhouse until December 28 when it was again stored in the refrigerator after its purity (as race 52) had been checked. It was again brought into the greenhouse in the spring of 1937, checked for purity and placed in storage on May 17. Part of the stored rust material was brought into the greenhouse once more in November, 1937. The infection types then produced on the differential wheat varieties indicated that race 52 had been largely replaced by another race, although the presence, on infected leaves of the variety Vernal, of a few large pustules characteristic of race 52 among the smaller pustules of some other race showed that the replacement was not complete. Attempts to recover race 52 by inoculating seedling wheat leaves with spores of these large pustules were unsuccessful owing to failure of infection. It is therefore not absolutely certain that the large pustules just referred to were actually those of race 52, although the likelihood is that they were. The remainder of the culture, on further study, proved to be a hitherto undescribed physiologic race with infection types differing widely from those characteristic of race 52, as is shown by the following comparison.

	LC*	Ma.	Krd.	Ko.	Arn.	Mnd.	Spm.	Kub.	Ac.	Enk.	Ver.	Kpl.
New race	4**	1	0;	0;	4	x−	x	x+	4	3+	0;	1
Race 52	4	4	4−	4	1=	1=	1=	x±	4	4−	4+	1−

* *Explanation of abbreviations:* LC = *Little Club,* Ma. = *Marquis,* Krd. = *Kanred,* Ko. = *Kota,* Arn. = *Arnautka,* Mnd. = *Mindum,* Spm. = *Spelmar,* Kub. = *Kubanka,* Ac. = *Acme,* Enk. = *Einkorn,* Ver. = *Vernal,* Kpl. = *Khapli.*

** *Explanation of symbols representing infection types:* 0; = *hypersensitive flecks,* 1 = *minute pustules surrounded by necrotic areas,* 3 = *pustules of moderate size,* 4 = *large pustules,* x = *pustules of various sizes on the same leaf.* (+) (±) (−) (=) *indicate variations in pustule size.*

In an attempt to recover race 52, inoculations were made four months later from the remaining spore material in storage in the refrigerator. The culture thus established proved to be a pure culture of the new race without any indication whatever of the presence of race 52. After some further study of the new race, its mean infection types were submitted to Dr. E. C. Stakman and Dr. M. N. Levine, who kindly numbered it physiologic race 178.

* *A selfing involves (i) inoculating plants of Berberis vulgaris with sporidia of a pure culture of a physiologic race, (ii) intermixing the nectar of the pycnial pustules, (iii) inoculating wheat plants with the aeciospores, and (iv) identifying the physiologic races in the uredial cultures thus established.*

The evidence for mutation in this instance rests on the facts (i) that the culture was placed in storage immediately after its purity as race 52 had been established, (ii) that while in storage there was no opportunity for contamination by another physiologic race, and (iii) that race 52 was replaced by a race not hitherto known. The fact that the original race was replaced by a race never before encountered appears to eliminate the possibility of contamination, which is the only alternative to mutation.

This instance of the replacement of one physiologic race by another suggests a series of recurrent, identical mutations similar to those described by Gassner and Straib (1) in *Puccinia glumarum*. It is clear, at any rate, that more than one urediospore was involved in mutation as the new race was obtained from the stored spore material on two separate occasions. The fact that race 52 was not recovered the last time the culture was re-established in the greenhouse does not, however, necessarily imply that all the stored spore material had become converted into the new race. The infection results obtained at that time suggested that only a small fraction of the stored spore material was viable. It is quite possible, therefore, that all the spores of the original race had been rendered non-viable by the long period of storage and that only a few spores of the new race remained germinable and capable of causing infection.

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ESTIMATION OF LEAF AREA IN WHEAT FROM LINEAR DIMENSIONS[1]

By J. W. Hopkins[2]

Abstract

Measurements of 80 to 90 leaves of each of four varieties of spring wheat at various stages of development indicate a fairly close statistical relation between area, and length and width, of the leaf blade. This relation was found to be essentially the same for all four varieties, and from a knowledge of length (L) and median width (W_M), the area of an individual leaf was given by the Least Squares relation log. A = 0.0094 + 0.934 log. L + 1.071 log. W_M, with a standard error of 4.2% of the antilog. Inclusion of a third measurement, width at three-quarters of the distance from base to tip (W_K), led to the relation log. A = −0.0438 + 0.970 log. L + 0.880 log. W_M + 0.189 log. W_K, giving estimated values having a standard error of 3.7% of the actual area per leaf.

This method of estimating leaf areas (i) is rapid in execution, and (ii) does not necessitate removing the leaves from experimental plants, which may accordingly be maintained intact for a series of physiological observations.

Introduction

In the study of certain types of plant physiological data, such as transpiration and growth rates, it is necessary to determine, or at least to estimate with reasonable accuracy, the leaf area of the experimental plants. It may be noted that it is customary to make comparisons relative to the area of the leaf laminae only, although in plants of the Grass family the leaf sheaths, which may attain considerable dimensions, are also functional in both transpiration and metabolism.

Four categories of methods for estimating the area of leaf laminae may be recognized. (i) Reproduction of the outline of each leaf by tracing, blueprinting, sensitized paper or similar means, and determination of the area thus delineated either directly or by weighing. This was the procedure employed by Brown and Escombe (1) in their classical studies of foliar metabolism, and is still used extensively. (ii) Use of an apparatus based on the photoelectric principle, the direct readings of which may be calibrated in terms of area. Problems and recent progress in this connection have been described by Kramer (3). (iii) By computation from the correlation between leaf area and leaf weight. This correlation is the basis of an indirect method of estimating the leaf area of field crops due to Watson (4). His procedure is to determine the mean leaf weight from a large random sample of plants, then to determine the leaf area : leaf weight ratio and its regression on leaf weight, from a relatively small subsample of single leaves. (iv) By measurement of linear dimensions, from which the area may be approximately computed. This was the method employed by Clements and Goldsmith (2) in their quantitative studies of ecological factors, using plants as measuring instruments. These authors conclude that the average area of both sides of a grain

leaf is 1.5 times the product of the extreme length and width, but do not indicate the order of accuracy to be expected from this approximation.

It will be appreciated that methods (ii), (iii), and (iv), are all more rapid than (i), but (ii) and (iii) both still retain the disadvantage that the leaves must be removed from the plant, which is consequently destroyed for further experiments. On the other hand, measurements of length and breadth, which may be made *in situ*, are not only equally rapid, but also leave the plant intact. As, however, the leaves of Grasses do not present regular geometrical figures, the relation between area and any specified linear dimensions must be a statistical one. The observations reported below were accordingly made in order to provide some information respecting the degree of precision to be expected in practice from the estimation of areas in this way.

Data and Results

Plants of four varieties of spring wheat, namely, Marquis, Reward × Caesium, Caesium × Marquis, and Lutescens, were grown in a greenhouse during the winter months from seed kindly supplied by Professor K. W. Neatby, of the University of Alberta. Specimens of each variety were collected at regular intervals for the measurement of length, width, and area of leaves at successive stages of growth. Length of leaf blade from base to tip was measured to the nearest $\frac{1}{2}$ mm., using a good quality steel ruler. Width at the mid-point, and also at a point three-quarters of the distance from base to tip, was measured to the nearest $\frac{1}{10}$ mm. by indicating calipers. Areas were determined by a Rotometer. In all, between 80 and 90 leaves of each variety were examined.

In general outline the wheat leaf is long and narrow, tapering to a point at the tip. As a rule, however, the width of the blade at first increases some-what as one proceeds upwards from the base until a maximum is attained which, in the case of full-grown specimens, is generally in the lower half of the leaf. The shape of the lower part of the blade is therefore approximately rectangular, or more correctly, trapezoidal, whilst the tapering upper part (neglecting the peculiar but characteristic constriction occurring at a point usually about $1\frac{1}{2}$–2 in. from the tip) presents approximately the aspect of an isosceles triangle, of which the base is small in relation to the perpendicular height. From geometrical considerations, therefore, a linear relation between area and the product of length and median width might be anticipated, which, however, would be expected to be only imperfectly realized in practice, owing to individual variations in the relative proportions of the trapezoidal and triangular portions of the leaf, to a certain degree of curvature in outline, and to occasional outright irregularities in shape.

Fig. 1, in which the measured area of 89 Lutescens leaves is shown plotted in relation to the product of length and median width, indicates that this is the situation actually prevailing.

Fig. 1 also illustrates the fact that irregular deviations are greater in the case of large than of small leaves. A logarithmic transformation tends to
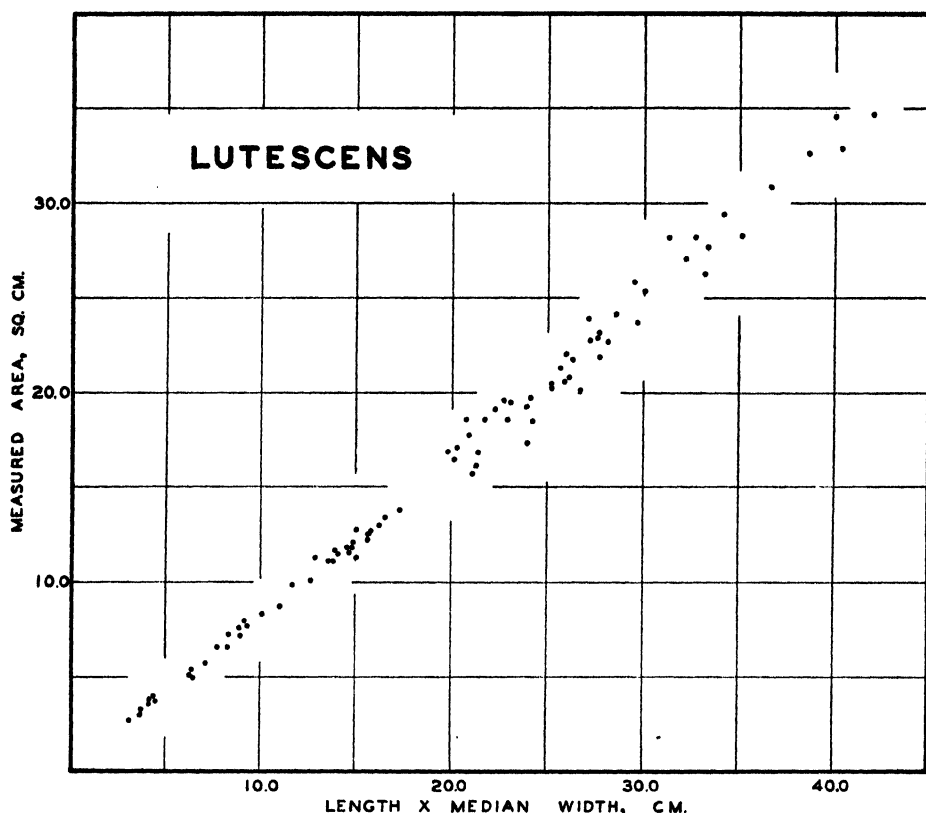
FIG. 1.

stabilize the variance in these circumstances, and also facilitates further analysis. The linear relation between area (A) and the product of length and median width $(L \times W_{\frac{1}{2}})$ was accordingly determined in logarithmic units for each of the four varieties, and was found to be:

Marquis: $\log. A = -0.0533 + 0.990 \log. (L \times W_{\frac{1}{2}})$
Reward $\times$ Caesium: $\log. A = -0.0560 + 0.986 \log. (L \times W_{\frac{1}{2}})$
Caesium $\times$ Marquis: $\log. A = -0.0652 + 0.995 \log. (L \times W_{\frac{1}{2}})$
Lutescens: $\log. A = -0.0290 + 0.984 \log. (L \times W_{\frac{1}{2}})$

The standard errors of estimation of log. A by these formulae are 0.0167, 0.0224, 0.0199, and 0.0197, respectively, or 3.9, 5.3, 4.7, and 4.7% of the antilog.

During the course of the measurements, it was observed that when the median width was above average, the point of maximum width tended to occur further up the leaf, resulting in an increase of the approximately rectangular relative to the triangular portion, and hence in a proportionately greater area.

This impression was confirmed by the computation of the regression of log. A on log. L and log. $W_{\frac{1}{2}}$ separately, which gave:

Marquis $\qquad$ log. A = $\quad$ 0.0106 + 0.932 log. L + 1.074 log. $W_{\frac{1}{2}}$

Reward × Caesium: $\quad$ log. A = $\quad$ 0.0105 + 0.936 log. L + 1.060 log. $W_{\frac{1}{2}}$

Caesium × Marquis: $\quad$ log. A = −0.0199 + 0.927 log. L + 1.118 log. $W_{\frac{1}{2}}$

Lutescens: $\qquad$ log. A = $\quad$ 0.0699 + 0.909 log. L + 1.073 log. $W_{\frac{1}{2}}$

The difference between the regression coefficients for log. L and log. $W_{\frac{1}{2}}$ is statistically significant, and as a result of taking this circumstance into account, the residual standard error of log. A is reduced to 0.0140, 0.0215, 0.0176, and 0.0179, or 3.3, 5.1, 4.1, and 4.2% of the antilog. for the four varieties respectively.

A still further increase in precision was obtained by utilizing the additional information provided by the width at three-quarters of the distance from the base to the tip of the leaf ($W_{\frac{3}{4}}$). When this was done, the Least Squares solutions for the regression equations became:

Marquis:

$\quad$ log. A = −0.0354 + 0.962 log. L + 0.921 log. $W_{\frac{1}{2}}$ + 0.153 log. $W_{\frac{3}{4}}$

Reward × Caesium:

$\quad$ log. A = $\quad$ 0.3580 + 0.794 log. L + 0.826 log. $W_{\frac{1}{2}}$ + 0.245 log. $W_{\frac{3}{4}}$

Caesium × Marquis:

$\quad$ log. A = −0.0479 + 0.965 log. L + 0.909 log. $W_{\frac{1}{2}}$ + 0.182 log. $W_{\frac{3}{4}}$

Lutescens:

$\quad$ log. A = −0.0030 + 0.954 log. L + 0.874 log. $W_{\frac{1}{2}}$ + 0.196 log. $W_{\frac{3}{4}}$

These give standard errors of estimation of 0.0120, 0.0187, 0.0157, and 0.0145 logarithmic units, or 2.8, 4.3, 3.7, and 3.4% of the actual area of an individual leaf.

In order to determine whether the inter-varietal differences in the regression coefficients of area on length and width were statistically significant, the standard errors of the individual coefficients were calculated. In the case of the first approximation, namely, the regression of area on the product of length and median width, the regression coefficients and their respective standard errors in logarithmic units, are:

$\qquad$ Marquis: $\qquad$ 0.990 ± 0.0045

$\qquad$ Reward × Caesium: $\quad$ 0.986 ± 0.0069

$\qquad$ Caesium × Marquis: $\quad$ 0.995 ± 0.0073

$\qquad$ Lutescens: $\qquad$ 0.984 ± 0.0074

The differences between the varietal coefficients are not significant in relation to their standard errors.

The coefficients and their standard errors (again in logarithmic units) in the case of the regression of area on length (L), median width ($W_{1/2}$) and width three-quarters of the distance from base to leaf-tip ($W_{3/4}$) are:

	L	$W_{1/2}$	$W_{3/4}$
Marquis:	0.962 ± 0.010	0.921 ± 0.030	0.153 ± 0.027
Reward $\times$ Caesium:	0.974 ± 0.017	0.826 ± 0.051	0.245 ± 0.039
Caesium $\times$ Marquis:	0.965 ± 0.016	0.909 ± 0.050	0.182 ± 0.039
Lutescens:	0.954 ± 0.016	0.874 ± 0.034	0.196 ± 0.029

There is no significant difference between the varietal regression coefficients in respect of either L or $W_{1/2}$. In the case of $W_{3/4}$, the coefficient for Reward $\times$ Caesium is slightly higher than those for the other three varieties, which do not differ significantly amongst themselves.

In these circumstances, it would seem that little additional error would be introduced by the use of an average regression equation. The data for the four varieties were accordingly pooled and used to determine such equations for two of the three approximations considered above, it having been found already that better results were obtained by the inclusion of L and $W_{1/2}$ separately than by the use of the product $L \times W_{1/2}$.

In this way, the average regression of A on L and $W_{1/2}$ was found to be:

$$\log. A = 0.0094 + 0.934 \log. L + 1.071 \log. W_{1/2}$$

with a residual standard error of estimation of A of 0.0182 logarithmic units, or 4.2% of the antilog.

Including $W_{3/4}$, the solution for the average regression equation was:

$$\log. A = -0.0438 + 0.970 \log. L + 0.880 \log. W_{1/2} + 0.189 \log. W_{3/4}.$$

This was found to have a residual standard error of estimation of A of 0.0158 in the logarithm, or 3.7% of the antilog., which may be regarded as satisfactory for the purpose in view.

The foregoing results cannot be compared directly with those of Clements and Goldsmith (2), but it may be noted that whereas they found 0.75 for the mean ratio of area to length $\times$ extreme width, the mean measured area of the 89 Lutescens leaves in Fig. 1 is 1619 sq. mm., and the mean product of length $\times$ median width 1960, giving a ratio of 0.86. It is evident, therefore, that the agreement in this respect must be fairly close.

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EFFECTS OF PLANT AND ANIMAL HORMONES ON THE ROOTING OF DUST- AND SOLUTION-TREATED DORMANT STEM CUTTINGS[1]

By N. H. Grace[2]

Abstract

Indolylbutyric acid and oestrone were applied in dusts to dormant stem cuttings of *Lonicera tartarica, Spiraea Vanhouttei*, and *Cornus alba*, and in both dusts and solutions to cuttings of *Ribes odoratum*. Indolylbutyric acid had significant effects on the number of cuttings rooted and the number and length of roots per rooted cutting of three species. Further observations indicated that it also affected the fresh root weight of cuttings of *Spiraea Vanhouttei* and the green leaf weight of *Ribes odoratum*. Oestrone had no significant effect on rooting, but in solution treatment showed significant effects on the green leaf weight of *Ribes odoratum*, both alone and in interaction with indolylbutyric acid. Cuttings of *Cornus alba* failed to show any significant treatment effects.

Dust and solution methods of treating cuttings were compared through the responses of *Ribes odoratum*. Dust treatment effected 62% rooting, solution 42%; there also was markedly greater leaf development following the use of dusts.

Oestrogenic substances have been shown to occur in plant materials, and their effects on plant growth have been reported by several investigators (1, 2, 4–10, 13). It has been concluded that some of these substances may have to be considered as plant hormones (11). While the influence of these chemicals on the rooting of cuttings has not been reported in detail, it is suggested that the number of roots produced by auxin treatment is increased by oestrogenic materials (12). The present communication describes the results of experiments in which dormant cuttings were treated by talc dusts and solutions of indolylbutyric acid and oestrone.

Experimental

Dust and solution preparations were compared, the indolylbutyric acid and oestrone being used at the same concentration; the interaction between chemicals was investigated by one series of solution treatments. Dusts containing 2000 p.p.m. of chemical in talc were prepared in a small laboratory ball mill. Subsequently, these were mixed with talc to give 1000 and 500 p.p.m. concentrations. Oestrone* solution was prepared by dissolving 0.0700 gm. in 3 cc. of warm 95% alcohol and making up to a volume of 350 cc., which gave a 200 p.p.m. colloidal solution. This solution was stirred continuously while aliquots were withdrawn. Indolylbutyric acid solution contained the same amount of alcohol.

Dormant cuttings of current year's growth of *Cornus alba* L., *Spiraea Vanhouttei* Zabel, *Lonicera tartarica* L. and *Ribes odoratum* Wendl. were

collected in mid-December, 1938*.   The cuttings of the first three species ranged from 10 to 12 in. in length; those of *Ribes odoratum* approximated 6 in., with two buds.   Treated cuttings were planted in brown sand in a propagation frame equipped with electrical bottom heat cables.   Sand temperature was maintained around 72° F., while the greenhouse temperature approximated 65° F.

The dust series of experiments contained seven treatments, namely, talc only, and 500, 1000, and 2000 p.p.m. of each chemical separately in talc. There were 10 cuttings to a group and 7 replicates of each treatment; 490 cuttings were required for each experiment.

Lonicera tartarica cuttings were left for 24 days in sand.   After determination of the number of cuttings rooted and the number and length of roots, the basal 2 in. of each cutting was removed and the entire experiment replanted in sand in random order, in the hope that residual treatment effects might be demonstrated.   The cuttings were examined 31 days later, to determine whether treatment with oestrone or indolylbutyric acid had an effect on the development of a second growth of roots by the shortened cuttings. Cuttings of *Cornus alba* were examined 28 days after planting.   *Spiraea Vanhouttei* cuttings were inspected 48 days after treatment.

Cuttings of one plant, *Ribes odoratum*, were treated with both dusts and solutions and were removed from the propagation frame 47 days after planting. The two experiments were placed contiguously in the same frame, thus permitting a rough comparison of the responses following treatment by two different methods of applying the chemicals.   The solution experiment was of factorial design, which permitted study of the interaction of indolylbutyric acid and oestrone.   There were four concentrations of the two chemicals, namely, 0, 10, 50, and 100 p.p.m.   This made a series of 16 treatment combinations, with 10 cuttings to a group and four replicates of each treatment.

The design of the experiments provided for analyses of variance of the observations.   Record was made of the number of cuttings rooted and the number and length of the primary roots formed.   The mean length of root was calculated.   In one experiment, where secondary root formation was pronounced, the roots were carefully washed in running water, placed between dry sheets of blotting paper for five minutes, and fresh root weights recorded. The new green growth produced by these dormant cuttings was also removed and weighed.

Results

Responses after Dust Treatment

In Tables I to III, data are given for responses after dust treatment with indolylbutyric acid and oestrone; residual effects are discussed separately.

Highly significant increase in rooting is shown by all three concentrations of indolylbutyric acid applied to cuttings of *Lonicera tartarica*, whereas the

corresponding concentrations of oestrone had no demonstrable effect. Only the means over the three concentrations of indolylbutyric acid and oestrone give significant results with *Spiraea Vanhouttei*; the indolylbutyric acid mean is significantly above that for oestrone but is not above the talc-treated control. Treatments failed to affect the number of *Ribes odoratum* cuttings rooted and the data are not given.

Oestrone treatments failed to have any effect on the number of cuttings rooted.

TABLE I

RESPONSES OF DORMANT *Lonicera tartarica* CUTTINGS DUSTED WITH PLANT AND ANIMAL HORMONES, AND RESIDUAL EFFECTS FOLLOWING REMOVAL OF BASAL ENDS

Data are means for seven groups of ten cuttings

	Talc dusted	Dusted with indolylbutyric acid in talc, p.p.m.			Dusted with oestrone in talc, p.p.m.			Necessary difference, 5% level
		500	1000	2000	500	1000	2000	
Number of rooted cuttings								
Transformed data*	1.92	2.86**	2.84**	3.06**	1.83	1.97	1.75	0.36
Untransformed data	3.4	7.7	7.6	8.9	3.0	3.6	2.7	
Number of roots per rooted cutting								
Transformed data*	1.99	3.08	3.53**	3.88**	2.05	1.98	2.00	0.45
Untransformed data	3.7	9.0	12.1	14.9	3.9	3.6	3.7	
Total root length per rooted cutting, mm.	63	245**	326**	337**	100	81	86	89

Residual effects

Number of rooted cuttings	6.3	4.3	5.6	5.1	5.9	5.0	3.9	—
Number of roots per rooted cutting	9.3	10.3	6.8	9.3	10.8	8.6	11.3	—
Total root length per rooted cutting, mm.	277	295	206	258	394	255	348	—
Mean length per root, mm.	30	30	27	26	36	31	30	—
Green leaf weight, gm.	6.2	6.0	6.4	6.1	7.7	6.7	6.5	—

* *Data transformed to* $\sqrt{x + \frac{1}{2}}$ *basis (3).*

** *Values significantly different from the talc dusted control.*

Treatment with indolylbutyric acid had significant effects on the number and lengths of root per rooted cutting in all species; oestrone treatment had no significant effect. Data on the mean length of root failed to attain significance for individual treatments. Partition of the treatment sum of squares for the data on *Ribes odoratum* indicated a lengthening effect over the mean of all treatments.

TABLE II

RESPONSES OF DORMANT *Spiraea Vanhouttei* CUTTINGS DUSTED WITH PLANT AND ANIMAL HORMONES

Data are means for seven groups of ten cuttings

—	Talc dusted control	Dusted with indolylbutyric acid in talc, p.p.m.				Dusted with oestrone in talc, p.p.m.				Necessary difference, 5% level
		500	1000	2000	Mean for all indolylbutyric treatments	500	1000	2000	Mean for all oestrone treatments	
Number of rooted cuttings										
Transformed data*	2.446	2.344	2.653	2.514	2.503	2.270	1.953	2.095	2.107	0.276†
Untransformed data	5.7	5.1	6.6	5.9	5.8	4.9	3.6	4.3	4.2	
Total root length per rooted cutting, mm.	380	508	711**	425	548	400	455	477	434	191 / 111†
Fresh weight of roots, gm.	1.04	1.74	2.54**	1.24	1.84	1.03	0.66	1.09	0.93	0.73 / 0.43†

* *Data transformed to $\sqrt{x + \frac{1}{2}}$ basis (3).*
** *Values significantly different from the talc dusted control.*
† *This necessary difference holds between means by chemicals; 0.39 is the necessary difference between control and group means.*

TABLE III

RESPONSES OF DORMANT *Ribes odoratum* CUTTINGS TREATED WITH PLANT AND ANIMAL HORMONE DUSTS

Data are means for seven groups of ten cuttings

—	Talc dusted control	Dusted with indolylbutyric acid in talc, p.p.m.			Dusted with oestrone in talc, p.p.m.			Necessary difference, 5% level
		500	1000.	2000	500	1000	2000	
Number of roots per rooted cutting	3.61	4.57	5.69**	7.07**	3.54	3.64	2.87	1.12
Total root length per rooted cutting, mm.	88	149	187**	254**	153	136	94	68.6
Mean length per root	25	33	32	35	42	36	33	
Mean for six treatments with two chemicals		35**						7.9*
Green leaf weight, gm.	2.2	2.5	2.1	1.4**	2.3	2.1	2.0	0.60

* *Necessary difference comparing talc dusted control and mean for the six dust treatments which do not vary significantly among themselves.*
** *Values significantly different from the talc dusted control.*

The 1000 p.p.m. treatment with indolylbutyric acid had a significant effect on the fresh root weight of *Spiraea Vanhouttei*; no other root weights were determined. No significant effects could be attributed to oestrone.

One significant treatment effect is shown by the green leaf weight of *Ribes odoratum* cuttings. The 2000 p.p.m. indolylbutyric treatment, which gave more roots per rooted cutting than any other, shows markedly reduced leaf growth. It would seem therefore that marked stimulation of the rooting response may be accompanied by reduced leaf development.

Cuttings of *Cornus alba* failed to show any significant treatment effects.

In the second part of Table I are given data on the residual effects of plant and animal hormone treatments on the various responses of *Lonicera tartarica* cuttings. While differences are suggested by the data, analyses of variance show that treatments are insignificant in each instance. Partition of the treatment sum of squares also failed to indicate any significant effects. It may be concluded therefore that removal of the basal end of the cutting, which takes away all roots on rooted cuttings and the zone which has been dust-treated, leaves a cutting whose further responses are not affected by the initial treatment.

Responses after Solution Treatment

In Table IV are given data for the responses of solution-treated *Ribes odoratum* cuttings. It is apparent that the 50 p.p.m. treatment with indolyl-butyric acid gives optimum rooting in the series, the response falling off at the 100 p.p.m. level. Indolylbutyric treatment also effects marked increase in both the number and length of roots per rooted cutting. Oestrone treatment, however, fails to show any significant increase in rooting over the

TABLE IV

RESPONSES OF DORMANT *Ribes odoratum* CUTTINGS TREATED WITH SOLUTIONS OF INDOLYLBUTYRIC ACID AND OESTRONE

Data are means for sixteen groups of ten cuttings

	Indolylbutyric concentrations, p.p.m. (average over all oestrone concentrations)				Oestrone concentrations, p.p.m. p.p.m. (average over all indolyl-butyric concentrations)				Necessary difference, 5% level
	0	10	50	100	0	10	50	100	
Number of rooted cuttings									
Transformed data*	1.86	2.08	2.36**	2.13	2.18	2.30	1.95	2.0	0.273
Untransformed data	3.2	4.1	5.2	4.2	4.3	4.9	3.6	3.8	
Number of roots per rooted cutting	4.1	3.9	6.2**	6.6**	4.9	5.6	5.2	5.1	1.32
Total root length per rooted cutting, mm.	113	143	203**	213**	148	177	182	166	61

* *Data transformed to $\sqrt{x + \frac{1}{2}}$ basis (3).*
** *Values significantly different from the respective control.*

control, although the 50 p.p.m. treatment is significantly below the 10 p.p.m. level; the interaction between indolylbutyric acid and oestrone is insignificant.

The data in Table V indicate that treatments have a highly significant effect on the weight of leaf produced. Both indolylbutyric acid and oestrone treatment, and the interaction between them, are significant. Leaf weight falls with increasing indolylbutyric concentrations, the values at 50 and 100 p.p.m. both being significantly below the 0 p.p.m. value. The 10 p.p.m. oestrone treatment is significantly above the 0 value, stimulating leaf development. There is significant depression at the 50 p.p.m. level and stimulation again as the 100 p.p.m. concentration is reached. The two stimulating concentrations, 10 and 100 p.p.m., do not differ between themselves, while the depressing 50 p.p.m. level differs significantly from the other three concentrations. The interaction effect would seem to be a consequence of the fact that both the stimulatory and depressive effects of oestrone are more pronounced at the 0 and 10 p.p.m. levels of indolylbutyric acid than when applied in conjunction with 50 or 100 p.p.m. of the latter substance.

TABLE V

GREEN WEIGHT OF LEAVES PRODUCED BY DORMANT CUTTINGS OF *Ribes odoratum* FOLLOWING TREATMENT WITH SOLUTIONS OF INDOLYLBUTYRIC ACID AND OESTRONE, IN GRAMS

Data are means for four groups of ten cuttings

Oestrone concentrations, p.p.m.	Indolylbutyric acid concentrations, p.p.m.				Means for oestrone treatments
	0	10	50	100	
0	1.03	0.73	0.70	0.43	0.72
10	1.50	1.15	0.80	0.58	1.01
50	0.65	0.40	0.58	0.35	0.49
100	0.88	1.18	0.90	0.60	0.89
Means for indolylbutyric treatments	1.01	0.86	0.74	0.49	
Necessary difference, 5% level: treatment means, 0.16; interaction, 0.32					

Comparison of Responses of Ribes odoratum Cuttings Following Dust and Solution Treatment

Incidentally, a comparison has been made of the dust and solution methods of treating cuttings through the responses of *Ribes odoratum*, averages over all treatments being considered. Since these two methods of applying physiologically active chemicals are in general use, comparison of the results is of considerable interest. In Table VI are given data for the responses following these two methods of treatment. Dust treatment effected 62% rooting, solution treatment 42%. This is of interest since the treatment effect failed to attain significance for the number of cuttings rooted after dust treatment. The absence of significant treatment effects after dust treatment may be attributed to the rooting of the talc-treated control. Solution treatment tends to produce somewhat more roots per rooted cutting. A striking difference is noted in the weight of green growth after dust treatment; this is

nearly three times that found on solution-treated cuttings. These differences would suggest somewhat greater physiological shock from solution treatment or a beneficial effect from the talc carrier.

TABLE VI

RESPONSES OF *Ribes odoratum* CUTTINGS FOLLOWING TREATMENT WITH DUSTS AND SOLUTIONS OF INDOLYLBUTYRIC ACID AND OESTRONE

Results for dust treatment are means of 490 cuttings, solution treatment means of 640 cuttings

	Dust method of treatment	Solution method of treatment
Number of rooted cuttings of 10 planted	6.2	4.2
Number of roots per rooted cutting	4.4	5.2
Total root length per rooted cutting, mm.	151.4	167.9
Mean root length, mm.	33.8	32.2
Green leaf weight, gm.	2.09	0.78

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VEGETATIVE PROPAGATION OF CONIFERS

II. EFFECTS OF NUTRIENT SOLUTION AND PHYTOHORMONE DUSTS ON THE ROOTING OF NORWAY SPRUCE CUTTINGS[1]

By N. H. Grace[2]

Abstract

Norway spruce cuttings were treated with phytohormone dusts, and nutrient solution was added to the sand in which some of the cuttings were planted. The nutrient treatment greatly increased the number of rooted cuttings and the number that developed new growth, and reduced the number that died. Although talc alone increased top growth, indolylacetic acid, present in three concentrations in talc, had no significant effect on the number of cuttings rooted or dead. However, the hormone dust treatment effected a significant reduction in the length of root per rooted cutting and the mean root length. The results indicate that nutrient salts may, under certain conditions, have a marked influence on the rooting and growth of Norway spruce cuttings.

While the importance of phytohormone chemicals in propagation is recognized, it is clear that other factors have a profound influence on the rooting of conifer cuttings. It has been demonstrated that the age of the tree, the position on the tree from which the cutting is taken, and the stage of development, are factors that affect rooting (2, 3, 7). Recent work by Nowasad has shown that nutrient solutions increase the rooting of alfalfa cuttings (5, 6). The present communication describes the results of an experiment at the National Research Laboratories, Ottawa, in which nutrient solution was applied to the sand in which a series of hormone-dust-treated Norway spruce cuttings were planted.

Experimental

The experiment was carried out in a propagation frame, which contained 5 rows of 12 glazed earthenware crocks provided with suitable bottom drainage and filled with washed brown sand over a layer of coarse sand. The crocks were set in sand heated by electrical cables.

There were five treatments:— untreated, talc only, and 5, 100, and 1000 p.p.m. of indolylacetic acid in talc. The experiment was arranged in the form of double blocks of 10 crocks. Each block contained two replicates of the dust treatments, one which received water only, the other nutrient solution weekly. The random arrangement gave the greatest precision to the comparison between nutrient and no nutrient treatments.

The nutrient solution used was Hoagland's; to it was added approximately 120 p.p.m. sodium chloride, 1 p.p.m. boron, 0.44 p.p.m. manganese and 0.16 p.p.m. zinc (4). Each of the 30 crocks receiving nutrient was given 200 cc. of this solution weekly for seven weeks. The solution was washed down

immediately with about 200 cc. of tap water. The rest of the crocks were given an equal amount of water at the same time. All the crocks were kept suitably moist by application of tap water as required. The pH of the nutrient solution was about 5.1, that of the sand immediately after treatment and washing down with tap water, 7.4. At the end of the experiment, four months after planting the cuttings, there was no significant difference in pH of the sand from crocks that had, and those that had not, received nutrient, the pH ranging around 8.1. The alkaline condition must be attributed to the Ottawa tap water, the pH of which ranges between 8 and 9.

Branches from the upper region of the tree were collected in January from a plantation of Norway spruce approximately 18 years of age and situated at the Dominion Forest Station, Chalk River, Ontario. The bases of the branches were covered with moist peat and held about two weeks in a room at 65° F. Cuttings were torn from the branches and trimmed with a knife, leaving a heel of old wood; they were divided into two classes, first, those from 10 to 20 cm. in length, and second, those from 5 to 10 cm. Each group of 15 contained 10 short and 5 long cuttings. There were approximately four laterals to one branch terminal. Groups of 15 were dust treated and planted immediately. The entire propagation frame was covered with a factory cotton screen. The temperature of the sand was maintained at 72° F. and that of the room between 65 and 75° F. However, after one month under these conditions, outside temperatures rose rapidly. It was impossible to keep down the room temperature which frequently rose to 90° F. or even higher during the latter part of the experiment.

Seven weeks after planting it was observed that most of the crocks were infected with an unidentified fungus. The incidence and extent of the infection did not appear related to nutrient treatment; nevertheless, this was discontinued. Each of the 60 crocks was treated with 100 cc. of a 2 p.p.m. ethyl mercuric bromide solution, and the treatment repeated twice at intervals of three days†. The organic mercurial application completely eliminated the infection and, apparently, had no detrimental effect on the cuttings.

Results

Cuttings were taken up four months after planting and a record was made of the number rooted, showing new growth, and dead, in each replicate of 15. The number of roots and their lengths were determined and the mean root length was calculated for cuttings that received nutrient. As only five cuttings rooted in the 30 crocks that did not receive nutrient, data on number and lengths of roots could not be analyzed for this part of the experiment. The data thus secured were subjected to analyses of variance, with the results indicated in Table I.

† *The ethyl mercuric bromide used in this experiment was prepared by a method developed in the Chemistry Division, National Research Laboratories, Ottawa, by Dr. A. Cambron, in the course of an investigation of the synthesis of alkyl mercury halides. The procedure followed yielded a product consisting of 80% ethyl mercuric bromide and 20% ethyl mercuric chloride.*

TABLE I

ANALYSIS OF VARIANCE OF RESPONSE OF NORWAY SPRUCE CUTTINGS TO PHYTOHORMONE DUSTS AND NUTRIENT SOLUTIONS

Source of variance	Degrees of freedom	Mean square					
		Number of cuttings‡			Mean, per rooted cutting		
		Rooted	Dead	With top growth	Number of roots	Length of roots, mm.	Mean root length, mm.
Replicates	5	76.60	43.52	46.30	0.225	1617.2	638.51*
Treatments	4	143.24	119.64	150.23*	0.280	2601.4*	577.47*
Error (a)	20	66.54	54.78	44.98	0.164†	696.1†	163.27†
Nutrient concentration	1	7898.24***	1847.04***	7346.05***	§	§	§
Interaction of treatments and nutrient	4	29.49	53.03	116.90	§	§	§
Error (b)	25	73.99	57.25	62.36			

‡ Data transformed from fractions to degrees (1).
† Eighteen degrees of freedom for error as two missing values have been estimated (8).
§ As only five cuttings rooted without nutrient treatment, data on number and length of roots could not be analyzed for that part of the experiment.
* Exceeds mean square error, 5% level of significance.
*** Exceeds mean square error, 0.1% level of significance.

The data in Table II compare the effects of nutrient and no nutrient on the cuttings rooted, showing new top growth, and dead. The upper half of the table gives percentages of the number of cuttings alive at the end of the four months in sand. The lower half gives percentages of the total cuttings planted. The data are presented in this manner because virtually 100% of

TABLE II

RESPONSES OF NORWAY SPRUCE CUTTINGS TREATED WITH NUTRIENT
SOLUTIONS, FOUR MONTHS AFTER PLANTING

	Treatment with	
	Nutrient solution	Water only
Living cuttings rooted, %..............	37.5	2.9
Living cuttings with new growth, %	31.8	1.2
Cuttings rooted, %†	20.7	1.1
Cuttings with new growth, %	17.6	0.4
Cuttings dead, %	44.9	61.8

† *Lower half of table gives percentages of all cuttings planted.*

the cuttings from 10 to 20 cm. in length died. It is apparent that weekly application of nutrient solution increased the number of cuttings that rooted and that showed new growth; there is, also, a highly significant reduction in the number of cuttings that died. It is of interest to point out that, of the nutrient-treated cuttings alive at the end of the experiment and without new growth, 38.5% were rooted, whereas 35.4% of the cuttings with new growth were rooted. Consequently, it is suggested that development of top growth by Norway spruce cuttings does not cause any marked reduction in the initiation of roots.

In Table III are given data for the effect of dust treatment on the number of cuttings with top growth, the length of root per rooted cutting, and mean root length. It may be seen from Table I that these are the only significant effects of dust treatment. Talc is significantly more effective than other

TABLE III

EFFECT OF HORMONE DUST TREATMENTS ON THE NEW GROWTH AND ROOT LENGTH OF NORWAY
SPRUCE CUTTINGS

	Untreated	Talc	Indolylacetic in talc, p.p.m.			Necessary difference, 5% level
			5	100	1000	
Number of cuttings having top growth, transformed data†	9.5	17.6	10.3	13.6	9.3	5.71
Per cent of planted cuttings showing new growth	6.7	15.6	5.6	10.6	6.6	
Root length per rooted cutting, mm.*	74.2	95.2	60.2	56.3	39.7	31.99
Mean root length, mm.*	44.0	54.0	41.7	44.7	26.8	15.50

† *Data transformed from fractions to degrees (1).*
* *Root lengths refer to cuttings which received nutrient solution.*

treatments in producing cuttings with top growth; the effects of other treatments differ only slightly among themselves. The greatest length of root per rooted cutting occurs on talc treatment, the results of which are significantly better than those of all three hormone dust treatments but not significantly better than those obtained with the untreated control. The 1000 p.p.m. level of indolylacetic acid reduces the mean root length. While the talc treatment gives the greatest mean length, the results do not differ significantly from those of the untreated control or the 5 and 100 p.p.m. levels. This reduction of mean root length of Norway spruce cuttings with a heel when treated with 1000 p.p.m. indolylacetic acid in talc has been reported previously (3). It is apparent that treatment with indolylacetic acid over the range from 5 to 1000 p.p.m. in talc has failed to stimulate the responses of Norway spruce cuttings; as little as 5 p.p.m. in the dust has reduced significantly the development of top growth.

The results indicate that nutrient treatment has a marked beneficial effect on the rooting of Norway spruce cuttings. In another experiment, the results of which will be published in due course, nutrient solution was used as the carrier for the hormone chemical but beneficial response was not indicated. It is possible that the high temperatures, the incidence of fungal infection, or initial storage of the branches in this experiment, are factors that accentuated damage and emphasized the effect of treating sand with nutrient. In consequence, it cannot be assumed that such marked stimulation of response would follow the use of nutrient treatment of the sand under growth conditions more nearly optimum. However, it is apparent that the judicious use of nutrient salts may present an important aid to the development of well rooted, vigorous conifer cuttings.

The absence of positive effects from indolylacetic acid treatment is noteworthy. The improvement from treatment with talc only is of interest and suggests that dusting of cuttings is advantageous. However, considerable work will be required before any definite mechanism for the effect of talc on cuttings can be established.

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RESPONSES OF DORMANT CUTTINGS OF *LONICERA TARTARICA* TO SOLUTIONS OF INDOLYLACETIC ACID AND NUTRIENT SALTS[1]

BY N. H. GRACE[2] AND M. W. THISTLE[3]

Abstract

Cuttings of dormant *Lonicera tartarica*, collected in March, were treated with a factorial series of indolylacetic acid and nutrient solutions. Indolylacetic acid was used at dosages of 0, 10, 50, and 100 p.p.m. in conjunction with 0, 1, and 10 concentrations of a modified Hoagland's nutrient solution. Indolylacetic acid treatment significantly increased the percentage of rooting, and the number and total length of roots, the fresh root weight and the green weight of leaf per group of cuttings treated, the higher concentrations having the greater effect. The use of nutrient also significantly affected each of the foregoing characters. The results suggest that some dormant cuttings may be deficient in minerals essential for rooting, and that there is an optimum nutrient concentration somewhere below the highest used in this experiment.

Recent communications describe the effects of treatment of dormant *Lonicera tartarica* cuttings with dusts containing indolylacetic acid, cane sugar and ethyl mercuric phosphate; and solutions of indolylacetic acid and cane sugar (3, 4). The present communication describes an experiment in which *Lonicera tartarica* cuttings, from the same collection of material, were treated with a series of indolylacetic acid and nutrient solutions. The experimental arrangement also permits a study of the interaction between the growth-stimulating chemical, indolyl-3-acetic acid, and a mixture of nutrient salts. This is of interest, since little has been reported on these interaction effects on plant cuttings, though it is known that their responses are affected by certain nutrient treatments (2).

Experimental

The experiment was of factorial design, with four concentrations of indolylacetic acid and three of a mixture of nutrient salts. Indolylacetic acid was used at 0, 10, 50, and 100 p.p.m. in solution. The nutrient solution was used at levels of 0, 1 nutrient, which contained in p.p.m., K 235, Ca 200, Mg 49, PO$_4$ 95, NO$_3$ 940, SO$_4$ 192, Na 47, Cl 73, B 1, Mn 0.44 and Zn 0.16; and 10 nutrient, which contained 10 times these amounts. This required a series of 12 treatment combinations, which were planted in four randomized blocks.

The entire experiment of four blocks of 12 treatments required 288 cuttings.

Stock solutions of indolylacetic acid and the mixture of nutrient salts were prepared (4). Each contained twice the amount of material required to give the highest treatment. These two solutions, by dilution and mixing, enabled the ready preparation of each individual concentration and mixture required. All the solutions were made with distilled water.

The cuttings were dormant material, 1938 wood, and were about 12 in. long. They were treated in groups of 24 cuttings (the four replicates) with 100 cc. of solution in 250-cc. beakers. The cuttings were held in solution at the laboratory temperature of approximately 72° F. for a treatment period of 24 hr. They were then rinsed and planted immediately in brown sand in a propagation frame equipped with bottom heat cables. The sand was maintained at about 72° F., while the room temperature dropped to 65° F. during the night and rose to around 75° F. during the day. However, during the last two weeks of the rooting period, day temperature frequently went up over 80° F. Further, this room had considerably greater light intensity than the one in which the other two series of *Lonicera* treatments were propagated (3, 4).

The cuttings were planted April 1 and removed for measurements May 9, 1939. A record was made of the number of cuttings rooted, the number and total length of roots, the fresh root weight, and the green weight of leaf for each group of six cuttings. In this experiment the use of 6 instead of 10 cuttings to a group, and rather indifferent rooting of the controls, made it desirable to make comparisons by groups rather than in terms of the individual rooted cutting. All the data were subjected to analyses of variance.

Results

The material dealt with in the present experiment proved to be highly variable, and much of the data appeared to be non-normal. Skewed data being unsuitable for the application of the analysis of variance, various schemes of transformation of the raw data were resorted to (1). The data on the number of cuttings rooted were treated by means of the inverse sine transformation, total root length data were transformed logarithmically, and the square root transformation was used on the data for the number of roots formed. The data on the weights of leaves and roots approximated normality, and were treated as they stood. For the purpose of direct comparison, treatment means of the untransformed data are presented in Table III.

It will be observed from Table I that there were significant treatment differences in respect of all five characters dealt with. Separation of the treatment components would seem to demonstrate the undoubted physiological activity of the nutrient solution employed, as well as a marked response to the use of indolylacetic acid. No significant interaction could be demonstrated, *i.e.*, there was no differential response at different nutrient levels to the same hormone dosage.

TABLE I

ANALYSIS OF VARIANCE OF RESPONSE OF *Lonicera tartarica* TO HORMONE AND
NUTRIENT SOLUTIONS

Source of variance	Degrees of freedom	Mean square				
		Number of cuttings rooted (transformed data)	Total root length (transformed data)	Number of roots (transformed data)	Green weight of leaves	Fresh weight of roots
Blocks	3	346.26	.88	17.91	2.08	.465
Treatments:	11	1809.19***	5.82***	55.26***	4.56***	1.354***
Indolylacetic	3	4621.49***	17.48***	152.96***	10.23**	3.361***
Nutrient	2	1837.09**	4.00†	39.87**	4.98†	1.044*
Interaction indolyl × nutrient	6	393.73	.61	11.55	1.59	.453
Error	33	265.38	1.29	6.48	1.55	.247

  * *Exceeds mean square error, 5% level of significance.*
 ** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*
  † *Mean square approaches significance. The two nutrient levels were thrown together, giving a comparison between nutrient and no nutrient. Resulting mean square exceeds 5% level of significance on remaining degree of freedom.*

Table II shows the effect of hormone and nutrient preparations on the number of cuttings rooted, total root length, number of roots, green weight of leaves, and fresh weight of roots per group.

In general, a significant increase is to be noted in all five criteria owing to the use of indolylacetic acid, the most pronounced effect being secured from 50 to 100 p.p.m. However, even the lowest concentration, 10 p.p.m., had

TABLE II

RESPONSE OF *Lonicera tartarica* TO HORMONE AND NUTRIENT SOLUTIONS

Treatment	Number of cuttings rooted (transformed data)	Total root length (transformed data)	Number of roots (transformed data)	Green weight of leaves, gm.	Fresh weight of roots, gm.
0 p.p.m. Indolylacetic	3	0.2	0.3	0.7	0.02
10 p.p.m. Indolylacetic	20*	1.7*	2.0	1.7*	0.20
50 p.p.m. Indolylacetic	41*	2.7*	6.2*	3.0*	1.14*
100 p.p.m. Indolylacetic	45*	2.8*	7.9*	1.9*	0.85*
Necessary difference for 5% level of significance	14	.9	2.1	1.0	0.41
0 Nutrient	16	1.3	3.0	1.2	0.30
1 Nutrient	37*	2.3 }*	5.9*	2.3 }*	0.81*
10 Nutrient	28*	1.8 /	3.4	2.0 /	0.55
Necessary difference for 5% level of significance	12	0.7	1.8	0.8	0.36

 * *Exceeds control 5% level of significance.*

significant effects on the number of cuttings rooted, total root length, and green weight of leaves, although not on the number of roots and fresh weight of roots.   The 50 and 100 p.p.m. means did not suffer significantly between themselves, except in respect of leaf weight, in which case the 50 p.p.m. mean was significantly higher than the 100 p.p.m. mean

In all cases the use of nutrient solution also produced a significant stimulation.   Of the two levels of nutrient employed, the evidence favours the lower, and the existence of an optimum concentration is strongly suggested.

TABLE III

RESPONSE OF *Lonicera tartarica* TO HORMONE AND NUTRIENT SOLUTIONS
Treatment means of untransformed data

Treatment	Per cent of cuttings rooted	Total root length, mm.	Number of roots
0 p.p.m. Indolylacetic	3	27	1
10 p.p.m. Indolylacetic	17	327	7
50 p.p.m. Indolylacetic	47	1951	54
100 p.p.m. Indolylacetic	50	1844	79
0 Nutrient	16	616	24
1 Nutrient	42	1787	60
10 Nutrient	30	709	22

Certain general comparisons may be made with the results of dust and solution treatments of the same dormant material, though the higher temperature and greater light intensity in which the cuttings of this experiment were propagated render it necessary to exercise care in making comparisons (3, 4).   It seems obvious, however, that this experiment has been carried out under sub-optimum conditions.   Either the higher temperature of the room, or the greater light intensity, or both these factors, had adverse effects on the responses.   Although these comparisons seem to indicate that nutrient salts had greater effects than sugar on the rooting responses of cuttings of dormant *Lonicera tartarica*, further experiments under identical conditions will be required before general conclusions can be reached.

Acknowledgment

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EFFECTS OF CANE SUGAR, ETHYL MERCURIC PHOSPHATE, AND INDOLYLACETIC ACID IN TALC ON THE ROOTING OF CUTTINGS[1]

By N. H. Grace[2]

Abstract

Cuttings of two herbaceous and two dormant woody plants were treated with a factorial series of talc dusts containing cane sugar, ethyl mercuric phosphate and indolylacetic acid. The effect of the dusts on cuttings of *Coleus Blumei* and *Iresine Lindeni* was determined by the number of roots per rooted cutting, the length of root mass and dry weight of roots. Each of the three factors gave at least one significant effect with both plants. *Iresine Lindeni* cuttings showed two significant interactions, one between organic mercury and indolylacetic acid on the number of roots per rooted cutting, the other between sugar and indolylacetic acid on the length of root mass. Dormant *Lonicera tartarica* cuttings showed significant effects from indolylacetic acid on the number of cuttings rooted, the number and length of roots per rooted cutting, the mean root length, and fresh root weights. Green leaf weights of this plant were significantly affected by sugar, and the sugar × organic mercury, and sugar × organic mercury × indolylacetic acid interactions. Fresh root weights also gave a significant triple interaction. The number of *Physocarpus opulifolius* cuttings rooted was significantly increased by organic mercury as were the dry root weights. Root weights also were affected by sugar treatment. This plant failed to make any significant response to indolylacetic acid treatment.

The results indicate that cane sugar and ethyl mercuric phosphate, as well as indolylacetic acid, affect some of the rooting responses of plant cuttings. It is suggested that the dust method of treating cuttings may be used to supply factors, other than the recognized growth stimulating chemicals, that are advantageous to successful vegetative propagation of plants.

The carrier dust method of applying root growth promoting chemicals to plant cuttings has been shown to be both effective and convenient (6). Preliminary work on the use of hormone dusts as a vehicle for nutrient salts indicated that improved rooting could be effected by this means. While numerous factors are known to influence the response of plants to growth stimulating chemicals, considerable work has been done on the effects of sugars and auxins (1–3, 5, 8, 10, 12–15, 17, 18). In consequence, detailed study has been directed first to hormone dust mixtures containing sugar. The effects of nutrient salts are under consideration also and will be reported later. The present communication describes the results of experiments in which cuttings were treated with a series of talc dusts containing indolyl-3-acetic acid, cane sugar, and an organic mercurial disinfectant, ethyl mercuric phosphate. Although organic mercurials are used extensively in seed disinfection, little is known as to their effect on the rooting responses of cuttings. The organic mercurial disinfectant was used in an effort to provide against the possibility of infections from the use of dusts containing large amounts of sugar.

Experimental

The effects of the three chemicals, cane sugar, ethyl mercuric phosphate, and indolylacetic acid, were investigated by an experiment of factorial design. The series of dust mixtures comprised cane sugar at five concentrations, namely, 0, 1, 5, 10, and 20%, in combination with ethyl mercuric phosphate at 0, 10, 50, and 100 p.p.m., and indolylacetic acid at 0 and 1000 p.p.m. The complete series of possible dosage combination of the three chemicals required the preparation of 40 different dusts. There were three replicates of the 40 individual treatments and one level of precision for all the different comparisons. This factorial arrangement is highly efficient in that it enables the use of the entire 120 groups of cuttings in determining the effect of each chemical or interaction.

The dust mixtures were prepared in talc by the grinding mix method (6). Firstly, dusts containing 10,000 p.p.m. indolylacetic acid and 1000 p.p.m. ethyl mercuric phosphate were prepared. These were then diluted with talc, and cane sugar was added directly to give the various concentrations and mixtures required. The source of the ethyl mercuric phosphate was a commercial seed disinfectant that contains 5% of the salt by weight. Analysis of the mercurial disinfectant disclosed that the P_2O_5 content was approximately 1.0%.* This means that dusts containing 10, 50, and 100 p.p.m. of ethyl mercuric phosphate carry approximately 2, 10, and 20 p.p.m. of P_2O_5 respectively. Preliminary experiments with the organic mercurial in talc indicated that 1000 p.p.m. had a markedly damaging effect on several varieties of plant cuttings; this damage was reduced but apparent at 500 p.p.m.; however, injury could not be detected visually when 100 p.p.m. or less was used.

Four varieties of plants were used in the investigation. Cuttings of two herbaceous plants, *Iresine Lindeni* Lem. and *Coleus Blumei* Benth., provided data on the effect of treatments on the number, mass length, and weight of roots. Since rooting was almost 100% for all treatments, these plants did not give information on the initiation of roots. Cuttings of *Iresine Lindeni*, 10 cuttings to a group, were treated and planted August 17, 1938, in an outdoor propagation frame containing brown sand, and covered with a factory cotton screen. The cuttings were taken up for measurements September 9, 1938. *Coleus Blumei* cuttings, 5 to a group, were planted in an identical frame August 20, 1938 and removed September 14, 1938. Two species of dormant woody cuttings were placed after treatment in an indoor propagation frame containing brown sand and equipped with bottom heat. The sand temperature was thus maintained at about 72° F., while that of the room was about 65° F. Both varieties had 10 cuttings in a group, and observations were made of the effect of treatments on the rooting of the cuttings, as well as effects on number and length of roots. *Physocarpus opulifolius* Maxim.

* *The analysis was carried out by Mr. C. W. Davis, Division of Chemistry, National Research Laboratories.*

cuttings* were treated October 20, 1938 and removed for measurements December 14, 1938. *Lonicera tartarica* L. cuttings were treated March 30, 1939 and removed May 1, 1939.

Freshly prepared cuttings were dipped in dust in groups of 10 cuttings (5 in the experiment with *Coleus*). The group was given a few quick turns in the dust, withdrawn, and shaken to remove excess. Treated cuttings carried a fairly uniform layer of dust over the cut ends and up the stem from the base for a distance of about 1 in. They were planted in fairly wide trenches in sand to avoid rubbing off appreciable amounts of the adhering dust preparations. The dusts containing 20% of cane sugar had a tendency to cake. However, the bottle was shaken and, if necessary, the dust poured out on paper and any cakes were pulverized with a spatula. Changes in dust consistency become more marked above the 20% level, and for this reason that concentration of sugar was chosen as the maximum.

The number of roots was counted on each rooted herbaceous cutting and expressed as the number of roots per rooted cutting for each group. One measurement was made of the length of the root mass of each cutting and expressed as mean length of root mass per group. While individual root lengths are to be preferred, the large number of roots rendered such measurements impossible in a reasonable time. Finally, dry root weights were determined for each group of cuttings. This was done by determining the difference in the weight of washed roots dried at 95° C., before and after ashing.

The number of dormant cuttings rooted per group was recorded and counts were made of the number of roots on each cutting. Total root length per rooted cutting was measured and mean root lengths calculated. Root weights were also obtained, dry weights for the roots of *Physocarpus opulifolius* and fresh root weights (7) for *Lonicera tartarica*. In addition, the weight of green leaf produced by each group of *Lonicera* cuttings was determined.

All the data for both herbaceous and dormant woody cuttings were subjected to analyses of variance.

Results

The results are presented in two parts; the first deals with the responses of herbaceous cuttings, the second with those of dormant woody material.

RESPONSES OF HERBACEOUS CUTTINGS

The results of analyses of variance of the data secured are given in Table I. It is apparent that indolylacetic acid had highly significant effects on the number of roots per rooted cutting and the dry weight of roots of both plants, but significantly affected the length of root mass of *Iresine* only. Sugar and organic mercury showed significant effects in at least one species on each of the three responses studied.

* *The prepared cuttings were supplied by the Federal District Commission, Ottawa, through the kindness of Mr. E. I. Wood.*

TABLE I

ANALYSIS OF VARIANCE OF RESPONSES OF HERBACEOUS CUTTINGS TO TALC DUSTS CONTAINING CANE SUGAR, ETHYL MERCURIC PHOSPHATE, AND INDOLYLACETIC ACID

Source of variance	Degrees of freedom	Mean Square					
		Number of roots per cutting rooted		Length of root mass, mm.		Dry weight of roots, gm. × 1000	
		Iresine	Coleus	Iresine	Coleus	Iresine	Coleus
Blocks	2	115.64*	99.56	118.86*	1182.21***	4.795***	23.109**
Indolylacetic acid dosage	1	2020.48***	4040.28***	3525.17***	61.20	6.873***	40.095***
Sugar dosage	4	88.27*	169.05*	26.38	344.11**	0.537	7.078*
Organic mercury dosage	3	94.20*	29.20	29.50	247.13*	0.522	0.940
Interaction Sugar × organic mercury dosage	12	31.40	35.63	21.24	60.66	0.181	1.669
Interaction Sugar × indolylacetic acid dosage	4	10.64	54.86	73.99*	66.67	1.008	2.191
Interaction Organic mercury × indolylacetic acid dosage	3	150.19**	68.19	10.83	59.33	0.348	2.211
Interaction Sugar × organic mercury × indolylacetic acid dosage	12	41.83	79.41	14.15	73.63	0.347	3.039
Error	78	29.45	47.66	25.83	88.82	0.458	2.653

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

Two significant interactions are to be noted, the first between organic mercury and indolylacetic acid dosage on the number of roots per rooted cutting of *Iresine*, and the second between sugar and indolylacetic acid dosage on the length of root mass of the same species. It is also apparent that the number of roots per rooted cutting was the measurement least affected by block variation; this observation has been noted on several occasions.

The treatment averages in Tables II and III indicate the nature of the main effects demonstrated by the analyses of variance. Averaged over all concentrations of sugar and mercury, treatment with 1000 p.p.m. indolylacetic acid increased the number and dry weight of roots of both plants, but decreased the length of root mass of *Iresine*. Sugar treatment increased the number, the length of root mass and weight of roots of *Coleus*; however, it decreased the number of roots per rooted *Iresine* cutting significantly at the 5 and 10% levels. Organic mercury decreased the length of root mass of *Coleus* and increased the number of roots per rooted *Iresine* cutting.

The data in Table IV show the average interaction effects over all sugar dosages of organic mercury and indolylacetic acid on the number of roots per rooted *Iresine* cutting. It is apparent that organic mercury alone failed to affect the number of roots significantly, though a slight reduction is suggested with increasing concentration. Indolylacetic acid alone did not

TABLE II

AVERAGE RESPONSES OF *Coleus Blumei* CUTTINGS TREATED WITH DUSTS CONTAINING CANE SUGAR, ETHYL MERCURIC PHOSPHATE, AND INDOLYLACETIC ACID

| | Effects of treatment with | | | | | | | | | | | | | |
| | Indolylacetic acid, p.p.m. | | Necessary diff., 5% level | Cane sugar, % | | | | | Necessary diff., 5% level | Ethyl mercuric phosphate, p.p.m. | | | | Necessary diff., 5% level |
	0	1000		0	1	5	10	20		0	10	50	100	
Number of roots per rooted cutting	19.5	31.1*	2.5	21.9	25.0	23.7	28.1*	27.8	3.9	60.4	58.6	56.6	53.8*	4.9
Length of root mass, mm.	51.9	28.1	1.9	51.9	58.3*	57.1	56.9	62.5*	5.4					
Dry weight of roots, gm.	0.072	0.109*	0.019	0.070	0.086	0.083	0.097	0.116*	0.030					

Significantly different from the corresponding 0 value.

TABLE III

RESPONSES OF *Iresine Lindeni* CUTTINGS TREATED WITH DUSTS CONTAINING CANE SUGAR, ETHYL MERCURIC PHOSPHATE, AND INDOLYLACETIC ACID

| | Effects of treatment with | | | | | | | | | | | | | |
| | Indolylacetic acid, p.p.m. | | Necessary diff., 5% level | Cane sugar, % | | | | | Necessary diff., 5% level | Ethyl mercuric phosphate, p.p.m. | | | | Necessary diff., 5% level |
	0	1000		0	1	5	10	20		0	10	50	100	
Number of roots per rooted cutting	25.5	33.7*	2.0	32.1	29.1	28.3*	27.5*	31.0	3.1	27.7	30.5	31.6*	28.53	2.8
Mean length of root mass, mm.	38.9	28.1	1.9											
Dry weight of roots, gm.	0.031	0.046*	0.004											

Significantly different from the corresponding 0 value.

TABLE IV

AVERAGE RESPONSES OF *Iresine Lindeni* CUTTINGS FROM THE INTERACTION OF INDOLYLACETIC
ACID AND ETHYL MERCURIC PHOSPHATE DOSAGE

	Indolyl-acetic acid, p.p.m.	Ethyl mercuric phosphate, p.p.m.			
		0	10	50	100
Number of roots per rooted cutting	0	26.2	27.4	24.9	23.4
	1000	29.3	33.6	38.3	33.6
Necessary difference, 5% level					3.96

increase the number of roots to a significant extent. However, significant increases followed combination with the three organic mercury levels, the maximum response being secured by the use of 50 p.p.m.

Table V shows the average effects over all mercury dosages of indolylacetic acid and cane sugar on the length of the root mass of *Iresine* cuttings. The only significant effect of sugar without indolylacetic acid was a reduction in length at the 20% level over that at the 1% concentration. Indolylacetic acid alone reduced the length markedly: with 20% sugar the length was significantly increased over the value at 10%. The 20% sugar concentration appears to protect the plant by decreasing the tendency to shorten the length of root mass.

TABLE V

AVERAGE RESPONSES OF *Iresine Lindeni* CUTTINGS FROM THE INTERACTION OF INDOLYLACETIC
ACID AND CANE SUGAR DOSAGE

	Indolyl-acetic acid, p.p.m.	Cane sugar, %				
		0	1	5	10	20
Length of root mass, mm.	0	40.6	41.0	37.4	39.2	36.8
	1000	28.4	27.9	27.9	25.1	31.1
Necessary difference, 5% level						4.2

RESPONSES OF DORMANT WOODY CUTTINGS

The results of analyses of variance of the responses of dormant woody cuttings to the various talc dusts are given in Table VI. Organic mercury had a highly significant effect on the number of rooted *Physocarpus* cuttings and on the dry root weights. These effects from treatment of cuttings with organic mercury are noteworthy. Sugar treatment had a significant effect on the root length per rooted cutting and on the dry weight of roots of *Physocarpus*. This plant, however, failed to show any significant

TABLE VI

ANALYSIS OF VARIANCE OF RESPONSES OF DORMANT CUTTINGS TO TALC DUSTS CONTAINING CANE SUGAR, ETHYL MERCURIC PHOSPHATE, AND INDOLYLACETIC ACID

		Mean square											
Source of variance	Degrees of freedom	Number of cuttings rooted		Number of roots per rooted cutting		Root length per rooted cutting		Mean root length		Root weights		Green weight of leaves
										Dry	Fresh	
		Physocarpus	Lonicera	Physocarpus	Lonicera	Physocarpus	Lonicera	Physocarpus	Lonicera	Physocarpus	Lonicera	Lonicera
Blocks	2	0.5799*	88.47	0.2194	3.15	1268	4128	658.2*	272.4	89	0.0711	35.71***
Indolylacetic acid dosage	1	0.0052	4034.48***	0.0210	105.28***	11900	429364***	270.0	2698.0*	2467	12.4936***	3.85
Sugar dosage	4	0.3142	35.78	0.1513	12.49	9247*	18982	115.3	189.1	3348*	0.4541	5.45**
Organic mercury dosage	3	0.9190***	30.34	0.0552	6.74	6722	14118	96.6	548.2	8550***	0.3571	3.64
Interaction Sugar X organic mercury dosage	12	0.1257	159.42	0.0775	6.59	3163	17784	156.9	222.5	1613	0.3963	2.77*
Interaction Sugar X indolylacetic acid dosage	4	0.2094	103.95	0.1431	4.49	2945	19788	141.7	380.9	857	0.4391	1.20
Interaction Organic mercury X indolylacetic acid dosage	3	0.1583	123.86	0.0535	0.67	2891	1893	81.2	680.0	1700	0.2835	1.29
Interaction Sugar X organic mercury X indolylacetic acid dosage	12	0.1575	141.01	0.0470	7.43	1127	24001	62.1	277.8	743	0.5643*	2.67*
Error	78	0.1474	95.46	0.0789	6.73	3578	14408	132.1	403.6	1307	0.2414	1.36

Exceeds mean square error, 5% level of significance.

**Exceeds mean square error, 1% level of significance.*

***Exceeds mean square error, 0.1% level of significance.*

TABLE VII

AVERAGE RESPONSES OF *Physocarpus opulifolius* CUTTINGS TREATED WITH DUSTS CONTAINING CANE SUGAR, ETHYL MERCURIC PHOSPHATE, AND INDOLYLACETIC ACID

Effects of treatment with

| | Ethyl mercuric phosphate, p.p.m. | | | | | Cane sugar, % | | | | | |
	0	10	50	100	Necessary diff., 5% level	0	1	5	10	20	Necessary diff., 5% level
Number of cuttings rooted—											
Transformed data†	2.28	2.61*	2.58*	2.68*	0.207						
Percentage rooting	50	65	63	69							
Root length per rooted cutting, mm.						183	176	221*	187	213	34
Dry root weight, mg.	67	90*	94*	108*	18	82	73	96	96	102	20

* *Significantly different from corresponding 0 value.*
† *Data transformed to* $\sqrt{x + \tfrac{1}{2}}$ *basis (4).*

response to indolylacetic acid treatment. *Lonicera*, on the other hand, showed significant responses to indolylacetic acid, the green weight of leaves produced being the sole exception; furthermore, in only one instance did the level of significance fail to attain the 0.1% point. Sugar had a significant effect on the weight of green leaf; this response, also, indicates significant interactions between sugar and organic mercury and sugar, organic mercury, and indolylacetic acid. Fresh root weights bring out another significant triple interaction. On the whole, the two experiments fail to show very significant block effects. However, the green weight of leaf of *Lonicera* cuttings shows a very highly significant block variation. This block effect appears to have been largely due to differences in light; the blocks receiving the greater intensity of light showed substantially more leaf production.

The significant responses of *Physocarpus* cuttings are given in Table VII. It is apparent that organic mercury increased the number of cuttings rooted at all three concentrations, which do not differ among themselves. On the average, marked stimulation of dry root weight resulted from organic mercury treatment, the 100 p.p.m. level being significantly above the 10, although not above the 50. Average dry root weights at 5, 10, and 20% of sugar were significantly better than that at 1% sugar, but were not significantly greater than the value at zero sugar. Sugar at the 5% level significantly increased the length of root per rooted cutting.

Average effects over all dosages of sugar and mercury of indolylacetic acid on *Lonicera* cuttings are given in Table VIII. Indolylacetic acid increased the percentage of cuttings rooted, the number and length of roots per rooted cutting, the mean root length and the fresh root weight. The substantial increase in mean root length is of interest, as solution treatment usually reduces the length if the effect is significant.

TABLE VIII

AVERAGE RESPONSES OF *Lonicera tartarica* CUTTINGS TO DUST TREATMENT WITH INDOLYLACETIC ACID

Data are means of 60 groups of 10 cuttings

Indolyl-acetic acid, p.p.m.	Number of cuttings rooted		Number of roots per rooted cutting	Root length per rooted cutting, mm.	Mean root length, mm.	Fresh root weight, gm.
	Trans-† formed data	Per cent				
0	35.21	34	3.4	127	37.6	0.345
1000	46.81	53	5.3	247	47.1	0.990

† *Data transformed to angles (4).*

In Table IX are shown the average effects of sugar treatment over both indolylacetic acid dosages, and the sugar × organic mercury interaction on the green weight of leaf of *Lonicera*. The 1% sugar mean does not differ

TABLE IX

AVERAGE GREEN WEIGHT OF LEAVES OF *Lonicera tartarica* CUTTINGS ON DUST TREATMENT WITH
CANE SUGAR AND ETHYL MERCURIC PHOSPHATE

Ethyl mercuric phosphate, p.p.m.	Cane sugar, %				
	0	1	5	10	20
0	8.10	8.17	8.02	6.12	7.25
10	8.29	7.60	6.22	6.27	7.57
50	8.52	8.40	8.27	7.22	7.38
100	8.23	7.55	7.18	8.65	7.67
Means for sugar treatments	8.28	7.93	7.42	7.06	7.47

Necessary difference, 5% level, interaction 1.34, treatment means 0.67

significantly from the control.   The 5, 10, and 20% sugar means are all significantly below the control, but do not differ significantly among themselves. The 10% mean also is significantly below the 1% mean.   The sugar $\times$ organic mercury interaction appears to be such that at the 0, 1, and 20% sugar levels, mercury had no significant effect.   At the 5 and 10% sugar levels, on the other hand, mercury had a detrimental effect at the 10 p.p.m., and at the 0 and 10 p.p.m. concentrations respectively.

In Table X are given the average effects of sugar and organic mercury on the response of leaf weights of groups of *Lonicera* cuttings to indolylacetic acid treatment.   The leaf weights are given in the form of differences between values obtained with and without the use of indolylacetic acid, permitting examination of the triple interaction.   At 0 and 5% sugar levels, the addition of 10 p.p.m. organic mercury produced significant reduction in response to indolylacetic acid, while at the 10% sugar level 100 p.p.m. organic mercury is necessary to produce injurious effects.   However, the 20% sugar level has significantly detrimental effects at zero organic mercury, and the addition of organic mercury reduced the effect to insignificance.

TABLE X

INTERACTION OF EFFECTS OF CANE SUGAR, ETHYL MERCURIC PHOSPHATE, AND INDOLYLACETIC
ACID ON THE WEIGHT OF LEAVES PRODUCED BY *Lonicera tartarica* CUTTINGS

	Ethyl mercuric phosphate, p.p.m.	Cane sugar, %				
		0	1	5	10	20
Differences in leaf weight, gm.	0	0.40	0.23	0.78	0.08	−1.38
(Differences between groups receiving and not receiving	10	−0.98	0.43	−0.78	0.33	0.07
	50	−0.45	0.30	0.13	−0.22	−0.22
indolylacetic acid)	100	0.13	−0.65	−0.08	−1.35	−0.37

Necessary difference, 5% level, 1.34

The average effects of sugar and organic mercury on the response of fresh root weights are given in Table XI. The fresh root weights are given in the form of differences between values obtained with and without the use of indolylacetic acid, permitting examination of the triple interaction on the fresh root weight of *Lonicera*. It appears that in the absence of mercury, indolylacetic acid has a significant effect at the 5% sugar level. With 5% sugar the addition of 10 p.p.m. organic mercury reduces the effect to insignificance, but the effect reaches significance again at the 100 p.p.m. concentration of organic mercury. With 10% sugar the addition of 10 p.p.m. organic mercury does not reduce the effect, but this does occur at the 100 p.p.m. organic mercury concentration. In other words the organic mercury effect reverses from 5 to 10% sugar levels.

TABLE XI

INTERACTION EFFECTS OF CANE SUGAR, ETHYL MERCURIC PHOSPHATE, AND INDOLYLACETIC ACID ON THE FRESH ROOT WEIGHT OF CUTTINGS OF *Lonicera tartarica*

	Ethyl mercuric phosphate, p.p.m.	Cane sugar, %				
		0	1	5	10	20
Differences in fresh root weights, gm. (Differences between groups receiving and not receiving indolylacetic acid)	0	0.13	0.16	0.81	0.58	0.11
	10	0.23	0.42	−0.14	0.81	0.21
	50	0.42	0.58	0.43	0.51	0.21
	100	0.34	0.11	0.70	−0.13	−0.05

Necessary difference, 5% level, 0.56.

A factorial series of dusts containing cane sugar, ethyl mercuric phosphate, and indolylacetic acid has also been applied to dormant cuttings of Norway spruce. Significant effects from all three factors were obtained on the length of root per rooted cutting and the interactions between sugar and indolylacetic acid, and sugar and organic mercury also were significant. These results will be given in detail shortly in a publication dealing with the vegetative propagation of conifers.

Conclusions

Each of the three chemicals, cane sugar, ethyl mercuric phosphate, and indolylacetic acid, had a significant effect on at least one of the responses of both herbaceous plants studied. Significant interactions of organic mercury and indolylacetic acid and sugar and indolylacetic acid were shown by *Iresine* cuttings. However, the response of both plants was not always the same to a given chemical. For instance, sugar treatment increased the number of roots on *Coleus* cuttings but decreased the number on *Iresine* cuttings. It must be pointed out that physiological activity has been determined by the

responses of cuttings that actually rooted, and it may not be inferred that all three chemicals will be effective in the treatment of herbaceous cuttings that root with difficulty. It seems reasonable, however, to expect that treatments which have a beneficial effect on the number of roots, the length of root mass, and the root weight will be of value in promoting the successful propagation of healthy vigorous plants from cuttings.

The dormant woody cuttings also showed significant effects from the three chemicals. Very highly significant effects from indolylacetic acid are shown by cuttings of *Lonicera*. The green leaf weights of this plant indicated significant sugar effects and an interaction between sugar and organic mercury and an interesting triple interaction; the latter was also shown by the fresh root weights. *Physocarpus* failed to respond significantly to indolylacetic acid in every case, but gave two highly significant effects from organic mercury and two less significant effects from sugar. The results from the two varieties of dormant woody cuttings consequently also suggest a measure of physiological activity for each of the three substances studied. The rather slight positive and detrimental effects from sugar suggest that dormant woody cuttings have an adequate reserve of carbohydrate material.

One of the most interesting features of the results is the physiological activity of organic mercury. Conflicting claims have been made for organic mercurials as stimulants to germination and the early growth of seedlings (9, 16). These results for the responses of cuttings certainly suggest physiological activity rather than mere fungicidal effects, though the latter may be a factor when sugar is present. However, it must be pointed out that a small but appreciable amount of phosphate is present along with the organic mercury. While it is unlikely that the marked activity of 10 p.p.m. ethyl mercuric phosphate can be attributed solely to the 2 p.p.m. P_2O_5 present, this possibility must be considered. Experiments now under way involve the use of organic mercurial preparations free from phosphate.

The results, as a whole, suggest that the dust method of treating cuttings may be used to provide accessory factors along with recognized growth stimulating chemicals such as indolylacetic or naphthylacetic acids. This conclusion is borne out by the results of Stoutemeyer, who has effected improved rooting through the addition of thiourea to treating dusts (11). Before optimum combinations and concentrations can be established, extended experimentation will be required with cuttings from a wide variety of plants, and with carbohydrates, nutrient salts, and other possible factors.

Acknowledgments

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RESPONSES OF DORMANT CUTTINGS OF *LONICERA TARTARICA* TO SOLUTIONS OF CANE SUGAR AND INDOLYLACETIC ACID[1]

By N. H. Grace[2]

Abstract

Cuttings of dormant *Lonicera tartarica*, collected in March, were treated with a factorial series of indolylacetic acid and cane sugar solutions. Indolylacetic acid was used at concentrations of 0, 10, 50, and 100 p.p.m., while cane sugar was present at 0, 1, 5, and 10%. Indolylacetic acid treatment greatly increased the percentage of cuttings rooted, the number and length of roots per rooted cutting, the fresh root weight and the green weight of leaf produced. Cane sugar treatment alone or in combination with indolylacetic acid failed to show any significant effects, suggesting that dormant cuttings of this plant have an adequate reserve of carbohydrate material.

Apart from a somewhat greater effect of treatment on the percentage of rooting, the results are in essential agreement with those previously secured from dormant October cuttings. In comparison with a parallel experiment on the dusting of March cuttings propagated in the same frame, solution treatment had the greater effect on all the responses considered except green weight of leaf produced, which was greater following dusting.

A recent communication describes the effects of treating *Lonicera tartarica* cuttings with dusts containing cane sugar, ethyl mercuric phosphate, and indolylacetic acid (2). Since there are some differences in the response of plant cuttings to the dust and solution methods of treatment, it was considered of interest to carry out an experiment in which cane sugar and indolylacetic acid were applied by solutions. The present communication describes an experiment that investigates the effects of both these factors and their interactions, using the solution method of applying the chemicals to the cuttings. The cuttings were from the same collection of material and propagated in the same frame as in the series of dust treatments mentioned (2). This fact permits a rough comparison of the responses of cuttings of this plant to the dust and solution methods of applying indolylacetic acid.

The responses of this March collection of *Lonicera tartarica* cuttings also are compared with those obtained with an October collection and treated with the same concentrations of indolylacetic acid (3).

Experimental

The experiment was of factorial design; cane sugar and indolylacetic acid were used at four concentrations, namely, 0, 1, 5, and 10% of the former, and 0, 10, 50, and 100 p.p.m. of the latter in solution. Indolylacetic acid solution was made by dissolving 0.2000 gm. of the pure chemical in 2 cc. of 95% alcohol and making up to one litre with distilled water, giving a 200 p.p.m. solution. A solution of 20% cane sugar was prepared by dissolving the commercial product in water. These two solutions, by dilution and mixing,

enabled the ready preparation of each individual concentration and mixture required. All the solutions were made with distilled water.

Each of the 16 possible combinations of indolylacetic acid and sugar was applied to four replicate groups of 10 cuttings. The cuttings (640 in all) were dormant material, 1938 wood, and were about 12 in. long. They were treated in groups of 40 cuttings (the four replicates) with 150 cc. of solution in 400-cc. beakers. The cuttings were held in the laboratory at approximately 72° F. for the treatment period of 24 hr. They were then rinsed and planted immediately in brown sand in a propagation frame equipped with bottom heat cables. The planting arrangement was in the form of four randomized blocks, each containing one replicate (10 cuttings) of each of the 16 treatments. The sand temperature was maintained at 72° F., while the room temperature ranged close to 65° F. The large dust series of *Lonicera* cuttings mentioned was present in the other end of the same propagation frame.

The cuttings were planted March 31, 1939, and removed for measurement May 5. Record was made of the number of cuttings rooted in each group, the number and length of roots per rooted cutting, the mean root length, fresh root weights, and the weight of green leaf produced. All the data were subjected to analyses of variance.

Results

In Table I are given results of the analyses of variance of the several observations made on the rooted cuttings. Highly significant effects from indolylacetic acid were shown by five of the six responses examined; the mean root length was not affected. Cane sugar treatments alone, or in interaction with indolylacetic acid, failed to show any significant effects. Furthermore,

TABLE I

ANALYSIS OF VARIANCE OF RESPONSES OF DORMANT *Lonicera tartarica* CUTTINGS TO SOLUTIONS OF CANE SUGAR AND INDOLYLACETIC ACID

Source of variance	Degrees of freedom	Mean square					
		Number of cuttings rooted	Number of roots per rooted cutting	Length of roots per rooted cutting	Mean root length	Fresh root weight	Green weight of leaves
Blocks	3	21.20	10.23	63565	464.43**	0.8500	4.703
Cane sugar dosage	3	26.04	19.01	11588	31.60	0.0689	1.115
Indolylacetic acid dosage	3	5101.05***	944.43***	971896***	158.68	46.6713***	41.265***
Interaction cane sugar × indolylacetic acid dosage	9	255.69	19.55	38502	149.95	0.8857	2.562
Error	45	132.74	19.01	29194	98.76	1.2548	2.570

† *Data transformed for analysis of variance, angular method (1).*
** *Exceeds mean square error, 1% level of significance.*
** *Exceeds mean square error, 0.1% level of significance.*

block variations are negligible except in the case of mean root length, in which they attain the 1% level of significance.

In Table II are given data (averages for all sugar dosages) for the significant responses of *Lonicera* cuttings to indolylacetic acid. The 10 p.p.m. mean is significantly above the 0 value, indicating an increase in the number of cuttings rooted. The 50 and 100 p.p.m. concentration means are significantly above both the 10 and 0 p.p.m. values, but do not differ significantly between themselves.

The 0 and 10 p.p.m. concentration means do not differ in their effect on the number of roots per rooted cutting. The 50 p.p.m. mean is significantly above both the 0 and 10 p.p.m. means but below the value at 100 p.p.m., which affects the number of roots per rooted cutting most markedly. Treatment effects are in the same order by concentration, for the length of root per rooted cutting, the 100 p.p.m. treatment giving significantly more length of roots than any other.

TABLE II

AVERAGE RESPONSE OF DORMANT *Lonicera tartarica* CUTTINGS TO SOLUTIONS OF INDOLYLACETIC ACID

Data are means of 16 groups of 10 cuttings

	Indolylacetic acid solution, p.p.m.				Necessary difference, 5% level
	0 ·	10	50 ·	100	
Number of cuttings rooted of 10 planted Transformed data† Per cent rooted	37.6 38.1	48.8 56.3	74.1 87.5	72.0 88.8	8.2
Number of roots per rooted cutting	5.1	5.0	13.1	21.1	3.1
Length of roots per rooted cutting, mm.	203	184	527	684	122
Fresh root weight, gm.	0.46	0.71	3.19	3.82	0.80
Green weight of leaves, gm.	5.37	5.61	8.81	7.31	1.14

† *Data transformed for analysis of variance, angular method (1).*

While the 100 and 50 p.p.m. concentrations both significantly increased the fresh weight of root, the values do not differ between themselves. There is no significant difference in the root weight at the 0 and 10 p.p.m. levels. In like manner the 50 and 100 p.p.m. treatments increased the green weight of leaf without differing between themselves, and the 0 and 10 p.p.m. concentrations do not differ.

The absence of significant effects from cane sugar on solution treatment of *Lonicera* cuttings suggests that some varieties of dormant cuttings have adequate reserves of carbohydrate material. It is of interest to point out that dust sugar treatments gave some significant effects, chiefly damaging in nature (2), as statistically significant effects were obtained by this method of treatment.

COMPARISON OF RESPONSES OF OCTOBER AND MARCH COLLECTIONS
OF *Lonicera tartarica* CUTTINGS

In Table III are given treatment averages expressed as an average of the control for the responses of October and March collections of dormant *Lonicera* cuttings to solution treatment with indolylacetic acid (3). This method of presenting the data reduces the various responses to a common level, and permits ready comparison of the effects of treatment. Treatment effects on the number and length of roots per rooted cutting and the mean root length are essentially similar for both October and March collections of dormant material. Treatments had a somewhat greater effect on number of cuttings rooted for the March collection. However, this fact is due largely to the greater rooting of the October controls, 59% against 38% for the March, a fact which may be due to the presence of 100 p.p.m. K_2HPO_4 rather than differences in the material at the two dates (3).

TABLE III

COMPARISON OF RESPONSES OF OCTOBER AND MARCH COLLECTIONS OF DORMANT *Lonicera tartarica* CUTTINGS TO INDOLYLACETIC ACID SOLUTIONS

Treatment averages expressed as average of control

| Month of collection | Indolylacetic acid solution concentration, p.p.m. | Cuttings rooted | Per rooted cutting | | Mean root length | Fresh weight of roots | Green weight of leaves |
			Number of roots	Length of roots			
October	10	1.16	1.01	0.90	0.84		
	50	1.42	2.97	2.45	0.81		
	100	1.59	4.25	3.29	0.80		
March	10	1.48	0.98	0.91	0.88	1.55	1.04
	50	2.30	2.57	2.60	0.99	6.92	1.64
	100	2.33	4.14	3.37	0.85	8.29	1.36

It is apparent from the data on number of cuttings rooted, for both collections, that there was slight difference between 50 and 100 p.p.m., indicating a levelling-off of effect. This tendency is not shown by the effect of treatment on number or length of roots per rooted cutting; with these responses the effects were markedly greater at 100 p.p.m. than at the 50 p.p.m. level. It is indicated that number and lengths of root per rooted cutting continued to increase with indolylacetic acid concentration even though the effect on rooting approached a constant level. It also is evident that a substantial increase in rooting can occur without marked effects on the number or length of roots. The weight of green leaf produced showed a maximum effect at 50 p.p.m.

COMPARISON OF EFFECTS OF SOLUTION AND DUST METHOD OF TREATMENT
ON RESPONSES OF DORMANT *Lonicera tartarica* CUTTINGS

Comparison of the results of this experiment with one in which *Lonicera* cuttings from the same collection were treated with dust preparations, indicates somewhat greater effectiveness for the solution method of applying

indolylacetic acid to this plant (2).   It may be seen that 1000 p.p.m. of indolylacetic acid in talc, over all dosages of sugar and organic mercury, effected 53% rooting, while 10 p.p.m. in solution rooted 56% of the cuttings. Solution treatment had the greater effect on all the responses excepting the green weight of leaf produced, which was 7.5 gm. per group of 10 cuttings on 1000 p.p.m. dust treatment, and 7.2 gm. on solution treatment, a mean of the values at the three solution dosages.   The results suggest that 1000 p.p.m. indolylacetic acid in talc (used nine months after preparation) had about the same effect as a 10 p.p.m. solution treatment.   A somewhat higher concentration of indolylacetic acid in talc would therefore appear to be required to give the rooting effected by solutions on this plant.   The relative effectiveness of the dust and solution methods of treating cuttings with growth stimulating chemicals would appear to vary with different plants, since *Ribes odoratum* showed dusts of indolylbutyric acid somewhat more effective than solutions (4).

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BUD DEVELOPMENT FOR THE FRUIT-BEARING SPUR OF THE WAGENER APPLE[1]

By Hugh P. Bell[2] and Jean W. McLellan[3]

Abstract

The complete development of the growing tip of the lateral fruit spur of the Wagener apple, from the time of its initiation until it produces mature fruit, requires four seasons. These are referred to as years I, II, III, and IV. The development during years I, II, and III may be divided into six typical phases. The growth during each of these phases is characteristic and different from that of any other stage. The phase just before initiation of the flower extends through June and the first half of July of year III ("off"). During this period the crown broadens and flattens, the pro-meristematic tissue becomes shallow, the scale and leaf primordium bases remain level with the crown and the pro-vascular strands and pith become broadly hemispherical. This phase is followed by flower formation, which is initiated during the last part of July, by the triangular, horizontal, upper surface of the crown becoming circular and developing five sepal primordia for the terminal flower. The flower cluster as a whole is "determinate", but its lateral flowers are axillary in origin and appear in acropetal succession. It is suggested that the changes occurring in the tip of the "off" spur during June, namely, the broadening and flattening of the crown, etc., may be an indication that physiological differentiation of the crown into flower-forming tissue is taking place.

Introduction

The investigation reported below was undertaken at the request of Mr. J. F. Hockey, Pathologist-in-charge, Laboratory of Plant Pathology, Kentville, Nova Scotia. The original purpose of the study was to determine for Kentville, Nova Scotia, the time during which the vegetative bud terminating the fruiting spur on the apple tree becomes differentiated into a flower bud.

Review of Literature

Date for the Earliest Initiation of Flowers

This is the first investigation of the subject for Nova Scotia, but a large number of investigators have reported on this point for other districts. Table I gives some of the dates that have been determined at other Stations for the earliest indication of flower bud formation.

It is evident that there is some variation in these dates, but it is reasonable to expect that there would be such a range, for there is a similar difference in the times for blossoming.

Criteria for Identifying the Earliest Stage of Flower Formation

Before the time of flower bud initiation could be determined, it was necessary to understand the morphological features by which the first stages could be recognized. Various descriptions of the first indication of flower formation

TABLE I

DATES FOR THE EARLIEST FORMATION OF FLOWER BUDS

Investigator	Date	Place	Reference
Goff	June 30	Wisconsin	8, p. 298
Drinkard	June 20	Virginia	6, p. 167
Kraus	Latter part of June	Oregon	16, p. 18
Bradford	First ten days of July	Oregon	4, p. 5
Magness	About the last of June	Oregon	18, p. 4
Kirby	About the first of July	Iowa	14, p. 265
Tufts and Morrow	June 11	California	22, pp. 7 and 9
Ranker	June 19	Utah	20, p. 411
Rasmussen	July 19	New Hampshire	21, p. 255
Gibbs and Swarbrick	June 25	Bristol, England	7, pp. 63 and 65

are as follows:—*irregular contour of, or corrugations on, the crown* (Goff; 8, p. 294: Drinkard; 6, p. 167); *thickening of axis and rapid elevation of crown* (Kraus; 15, p. 5: Bradford; 4, p. 5: Magness; 17, p. 53: Rasmussen; 21, p. 258); *a flat crown* (Gibbs and Swarbrick; 7, p. 63); *a broad crown* (Kirby; 14, p. 274: Tufts and Morrow; 22, p. 6: Aaron; 1, p. 259). It is impossible to decide which author should be followed, for apparently they are not all describing the same structure. Also, from a study of the literature it is evident that most of the investigators take as the initial indication of flower formation, structures which are definitely flower or flower cluster primordia. By the time such a stage is reached the whole question of differentiation is finalized, and the tip is as certainly a flower bud as when the young flowers are completely formed some months later. It was felt that it might be possible to find some early stage in development, before the actual morphological appearance of the flowers, which would distinguish the potential flower-forming tip from the purely vegetative tip. Thus an intensive study of the development before and including the actual appearance of the flower was undertaken.

Length of Period During Which Flower Bud Initiation Occurs

In regard to the period during which flower buds may become differentiated, it is necessary to know not only the time when it starts but how long it lasts. On this there is disagreement among the authorities, some (Ranker, 20, p. 411; Gibbs and Swarbrick, 7, pp. 64 and 65) being of the opinion that flower bud initiation takes place at the first of the summer only and that the time during which it may occur is comparatively short and limited. Other investigators (Goff; 10, p. 311: Drinkard; 6, p. 204: Kraus; 16, p. 18: Bradford, 4, p. 5: Gourley; 12, p. 5: Magness; 17, p. 55: Kirby; 14, p. 265) consider that the initiation of flower bud formation continues all summer and even into the autumn. In addition, there are opinions given (Tufts and Morrow; 22, p. 10: Rasmussen; 21, p. 260) which are intermediate between these two extremes. So many investigators pronounced in favour of the protracted period, that the material collected during the latter part of the summer was examined very carefully to see if there was any evidence to support this view.

Structural Units of the Growing Tip

The leaves of the apple tree are produced in a spiral with a phyllotaxy fraction of two-fifths. The growth process which gives rise to such a structure is really quite complicated and a complete description must include detailed information on each of the morphological units of which the growing tip is composed. For convenience of reference these will now be listed and numbered under headings and sub-headings. *(1) The "crown" or pro-meristematic tissue at the tip.* To treat this structure adequately it must be studied from the following standpoints:—*(a), shape of the horizontal upper surface as seen in transverse section; (b), the contour of this surface as seen in longitudinal section; (c), the width of this surface and (d), the depth of the pro-meristematic tissue. (2) The leaf primordia and young leaves. (3) The pro-vascular tissue. (4) The cells developing into pith tissue.* Each of these structural units will be discussed under the headings as listed above.

FIG.1   FIG.2   FIG.3

FIG 4   FIG.5   FIG.6
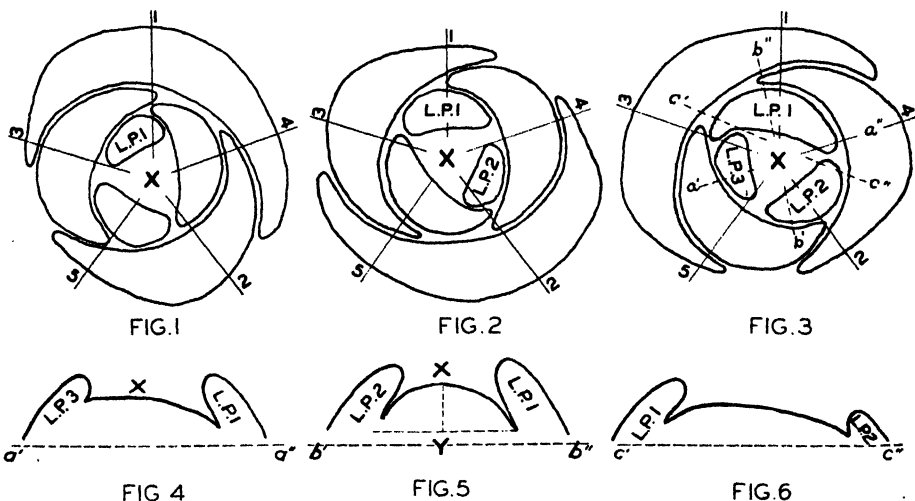
FIGS. 1 – 6. FIGS. 1, 2, AND 3. *Diagrammatic horizontal projections of leaf primordia at the level of the crown-surface in a vegetative bud. These diagrams represent three successive stages in development. The leaf primordia are numbered according to their genetic sequence. The phyllotaxy of two-fifths is indicated by the radial lines 1 to 5. L.P., leaf primordium; X, centre of crown.* FIGS. 4, 5, AND 6. *Enlarged longitudinal sections through $a'-a''$, $b'-b''$ and $c'-c''$ (Fig. 3) respectively; L.P., leaf primordium; X, centre of crown; $X-Y$ (Fig. 5) distance which the base of leaf primordium No. 3 is above the bases of leaf primordium No. 1 (Fig. 3).*

1a. Shape of the Crown as Seen in Transverse Section

The shape of the horizontal upper surface of the promeristem is roughly an equilateral triangle, but it is very difficult to describe this area, for it is constantly undergoing changes which are varied, complex and continuous. In Figs. 1 to 3 this growth process is illustrated by horizontal projections of the tip in three successive stages, but the best way to represent it is to make a model of the tip at any one stage and then rotate this model through 720 degrees, stopping at each of the five points 144 degrees apart (axes 1, 2, 3, 4,

and 5, Figs. 1 to 3). The model when stopped at each of these five points will illustrate the progressive development of the tip. However, in spite of the many changes that occur over the surface of this crown, there is one feature which is constant and characteristic of purely vegetative growth, that is, the roughly triangular outline of the horizontal upper surface.

1b. Contour of the Crown as Seen in Longitudinal Section

For any one growing tip the contour of the upper surface depends upon the plane in which the longitudinal section is cut. Figs. 3 to 6 illustrate this point. It will be seen that longitudinal sections through $a'-a''$ and $b'-b''$, Fig. 3, would both be median, but $a'-a''$ would have a contour as shown in Fig. 4, whereas the tip through $b'-b''$ would appear as illustrated in Fig. 5. An extreme and incorrect conception of the tip would be obtained by a section through $c'-c''$, Fig. 3, when the tip would appear to have a broad flat crown as indicated in Fig. 6. Thus, depending on which median plane is used and whether the plane of the cut is median or slightly lateral, the one growing tip might give a great variety of outlines for the external surface of the crown as seen in longitudinal section.

The sections most useful for indicating the characteristic contour were the median ones. In this work these significant sections were determined by counting the number of sections that included the pro-meristematic tissue of the crown. Then those chosen as most likely to represent the true centre of the bud were the central numbers of the series. If there were 17 sections showing pro-meristematic tissue, Nos. 8, 9, and 10 were considered as significant or central for that growing tip. It is evident that a continuous and complete series is absolutely necessary for such a determination. Failure to follow this procedure led to grave errors. Hence, unless otherwise stated, all drawings of growing tips in the present paper are from sections selected as described above.

Respecting the information that can be gained from a study of these contours, the median longitudinal section cut through the plane $b'-b''$, Fig. 3, and giving the usual convex outline illustrated in Fig. 5, is perhaps the most significant. For the central point, "X" (Figs. 1 to 5) is always on a level with the base of the youngest leaf primordium (see L.P. 3; Fig. 4); thus the distance X–Y, Fig. 5, represents fairly well the amount of new growth at the tip since the formation of leaf primordium No. 1. Hence a decidedly convex crown in such a section suggests rapid growth, and conversely a flatter crown in the same type of longitudinal section suggests a slowing down of growth in length. This is by no means an infallible guide, but it is helpful. Due to the significance of this type of section, the terms "convex", "flat", etc., when used in the present article, apply to the contour of the crown as seen in a median longitudinal section through a plane similar to $b'-b''$, Fig. 3.

1c. Width of the Crown

There are frequent references in the literature to the "width" of the pro--meristematic area, but what is the width of a triangle the outline of which

is not continuous? In some articles this width is indicated in microns by a figure which was arrived at by making one measurement for each tip. Any attempt to indicate the width of the crown by one such measurement may be misleading. What is required is not the size of the crown in one growing tip, but a means by which the size of the crown in one tip may be compared with the size of the crown in some other tip. It was found that the most accurate indication of comparative sizes was obtained from the average of three measurements. This average is called the "Index of Crown Width". To obtain the necessary data to compute this index, complete series of longitudinal sections were used and three measurements taken as follows: (i) by multiplying by ten the number of sections showing pro-meristematic tissue (the sections were cut 10μ thick); (ii) by using the median section of this series and measuring in microns the distance from the youngest primordium base of one side to the youngest primordium base of the opposite side; (iii) by using the section through that tip which showed the greatest pro-meristematic width, and making for it a measurement similar to that made in (ii). The average of these three figures was then considered as the "Index of Crown Width" for that bud. The index given for each week is the average of the indices of all the buds collected during that week. The number of buds examined for each week ranged from two to eight. This index must not be considered as an accurate representation of size, but merely a comparative indication.

Instead of using the crown width index as an indication of crown size, an attempt was made to use the area of the upper surface of the pro-meristematic region and to compute this area from transverse sections, but it was impossible to decide when the youngest primordium should be included in the area and when it should not. There is no sudden change in the natural development from unquestioned terminal pro-meristematic tissue to definite and distinctly marginal primordia. Any arbitrary division led to misconceptions, so this plan was abandoned.

1d. The Depth of the Pro-meristematic Tissue

The vertical depth of the pro-meristematic tissue at the tip varies greatly from season to season. The measurements for this were made in the median longitudinal section and represent the distance in microns from the outer surface of the centre to the first cells showing definite vacuolation, preparatory to enlarging into pith cells. The inner limits for this measurement are obviously indefinite, but the figures will be an aid in comprehending the changes in structure at the tip.

2. Leaf Primordia and Young Leaves

In studying the leaf primordia and young leaves the most important point to note is the position of their bases in respect to the level of the surface of the crown, that is, whether many of the bases are on the same level with the pro-meristematic tissue or most of them lower down. Of those that are lower down, the vertical distance between the bases affords an excellent indication of the rate and extent of growth.

3 and 4. Pro-vascular Tissue and Pith

With both the pro-vascular tissue and the pith, the significant feature is the outline of the distal portions as seen in median longitudinal section. The terms used for describing this are, "acute conical", "broad hemispherical", and "narrow cylindrical", etc. These are self-explanatory.

Distinction Between Vegetative Buds and Flower-forming Buds

To interpret the early stages correctly, it was of paramount importance to be able to differentiate potential flower buds from those that were potentially vegetative only. During the first part of this investigation, all collections were made from the McIntosh Red. The bearing habits of this variety are such that one cannot always foretell with accuracy which buds will remain vegetative and which will develop into flower buds. The only way to secure buds that could be classified in this way, with a minimum chance of error, was to have trees on which, during one season, the main bud of the fruit spur was of one type only. Ordinary "off" and "on" trees (so called) would not be suitable, for most varieties which are casually termed "biennial bearers" really have a heavy crop followed by a light crop. A search of the records of the orchard showed that certain Wageners were very heavy bearers every second year and really completely "off" during the alternate years. The two most suitable Wageners were selected, and by taking buds from these trees only, the possibility of error at the time of collection was reduced to a minimum.

When these collections were made, buds were not taken indiscriminately, but the data provided by such workers as Crow (5), Maney and Plagge (19), and Hooker (13) were taken into consideration and all collections were made from spurs of medium length. Also, when collecting from the "off" tree, buds were taken from spurs that during the previous year had produced a medium amount of growth and had actually borne fruit. Similarly, when collecting axillary buds from the "on" tree, long whip-like or stunted growths were avoided, and material was taken only from spurs actually bearing blossoms or fruit. In this way it was hoped to limit the study as far as possible to growth that would have produced fruit in the normal biennial cycle.

Methods and Technique

During this investigation various methods were employed. Firstly, both longitudinal and transverse sections in series were obtained. Using a complete longitudinal series through a bud, camera lucida drawings of each section were made on sheets of wax. These drawings were cut out and built into models. As the wax had been poured to a predetermined thickness, correct proportions were obtained for the models. These models were of assistance, but the greatest aid was to dissect, stain and examine the young tip in the way described by Gore (11). To get a satisfactory dissection it was necessary to use the special needles described by Henderson (Bell and Facey, 2. pp. 130–131) and to soak the bud for 24 hr. in 70% alcohol before dissecting. Fresh buds were too soft to dissect and pure alcohol made them too brittle.

Just before completing the final stages of the dissection, the bud had to be stained in the tincture of iodine, otherwise the smallest leaf primordia could not be seen clearly and might be injured by the needles. The completely dissected tip, stained in tincture of iodine, placed in a ray of very strong light and examined under a magnification of at least 150 diameters, revealed details of structure which could be seen clearly by no other method. For microtome work, material was killed, imbedded, and sectioned as described by Bell and Facey (2). The sections were cut 10 μ thick. The stain that proved most satisfactory was a combination of safranin and fast green.

FREQUENCY OF COLLECTIONS

With both the McIntosh and Wagener, the collections were made at least twice each week from April to October inclusive, twice a month during November and March, and once a month during December, January, and February. For each collection an average of 12 buds was taken from scattered parts of the tree. The collections of McIntosh were started in April 1934, but discontinued late in the summer of 1936. The collections of Wagener were continuous from April 1936 to September 1938.

The Development of the Growing Tip

Explanation of Terms Used

A short explanation is necessary regarding the terms used in the following description of development. Firstly, the mature apple is the climax and end of a growth that was initiated over three years prior to the date on which the apple is picked. It is obvious that developmental cycles overlap and at any time more than one stage may be found on one tree. To avoid confusion, the development of one tip will be followed through from start to finish. It would be misleading to use dates. To avoid their use, the season during which the tip first becomes differentiated as a separate organ will be referred to as year "I", the next year as "II", and so on. To correlate the symbols I, II, etc., with the terms "off" and "on", year I is an "off" year; II, "on"; III, "off"; and IV, "on". To make the description clearer, the development will be dealt with in what appeared to be natural "phases", but this is not meant to indicate that there is any sharp transition between one phase and the next, for of course development is continuous.

PHASE 1. Initiation of a New Growing Tip

From summer of year I ("off") to winter of years I ("off") and II ("on"). Figs. 10 and 18a.

Year I, for the particular spur under discussion, is an "off" season. During the summer of this year I, there is matured at the terminus of the spur a mixed bud containing the primordia of about five flowers surrounded by five to seven leaves. As usual, in the axil of each young leaf, is a small group of embryonic cells (potential growing tips). Towards the end of the summer, the embryonic tissue in the axil of at least one of the leaves (which is usually

TABLE II
INDEX OF CROWN WIDTH AND DEPTH OF PRO-MERISTEMATIC TISSUE

Year II, "On"			Year II, "On"—*Concluded*		
Collection date	Index of crown width	Depth of pro-meristematic tissue, μ	Collection date	Index of crown width	Depth of pro-meristematic tissue, μ
April 29	88	113	Nov. 4	76	124
May 2	95	113	19	94	128
4	111	94	Dec. 10	72	134
5	70	101	Year III, "Off"		
6	74	103			
7	121	94	Collection date	Index of crown width	Depth of pro-meristematic tissue, μ
8	95	106			
9	120	99			
14	124	97	Jan. 20	111	129
18	104	103	Feb. 5	83	137
21	149	105	Mar. 5	129	149
25	123	110	19	104	142
28	135	99	April 2	87	134
June 1	124	113	12	102	142
4	153	101	21	97	135
6	144	97	28	92	124
8	143	90	29	80	103
11	108	85	May 2	105	94
13	142	89	4	121	92
15	163	76	5	108	112
18	179	86	6	100	97
20	149	97	8	120	106
22	158	83	11	129	106
24	124	83	14	121	88
25	106	71	18	121	103
27	164	97	19	87	80
29	138	93	21	100	80
July 1	136	87	25	115	89
2	158	83	26	115	80
4	159	112	28	170	97
5	181	71	30	127	84
6	141	84	June 1	199	97
7	143	101	4	203	79
8	110	71	6	145	84
11	144	84	8	209	97
12	184	80	11	142	80
13	143	93	13	167	93
15	132	100	15	134	84
18	114	97	18	143	86
19	152	103	20	207	89
22	136	89	22	148	89
26	129	89	25	200	89
27	145	89	27	165	85
29	136	89	29	155	71
Aug. 1	134	94	July 2	210	97
2	161	93	4	158	89
5	131	97	6	164	97
8	147	88	7	151	93
15	120	80	11	152	101
22	112	92	13	152	97
29	118	94	15	134	97
Sept. 3	121	93	18	145	95
15	139	103	20	153	88
20	101	112	22	164	103
Oct. 4	93	127	25	197	130
20	102	119	27	182	128
			29	171	101

neither the highest nor the lowest) has developed into a definite growing tip with at least two leaf primordia of its own (Figs. 10 and 18a). It is the development of this tip which will be traced from its inception during the the summer of year I until its individuality as a growing tip ceases, with the formation of flowers. By the end of August, year I, this young growing tip is quite easily identified with the aid of a magnifying glass. In composition its cells are practically all embryonic, those of the young leaves being differentiated only slightly. The crown is narrow and convex. In this condition it overwinters during the winter of years I and II.

PHASE 2. Development of a Broad Crown in the Vegetative Bud

From the early spring of year II ("on") to late July of year II ("on"). Figs. 11, 19, and 20a.

When activity of this axillary growth starts in the spring of year II its crown is at first narrow and decidedly convex. The pro-meristematic tissue is medium in depth and neither the pro-vascular strands nor the pith are distinctly differentiated. Before the expansion of the blossoms, this new growth is enclosed within the flower bud and is difficult to locate without the aid of a magnifying glass. During the latter part of May it grows in length, and by the time of full bloom it may be seen as a very short lateral branch, emerging from the axil of one of the leaves (Fig. 19). The leaves formed by this new and axillary growing tip become matured just after those which surround the flower cluster. This new growth is then established as one of the gross morphological features of the spur.

By this time (the middle of June) a number of changes have taken place in the growing tip of this new lateral branch. The crown has broadened, though it is still convex. The leaf primordia are below the surface of the crown. The pro-meristematic tissue is not as deep as it was, but is still of medium depth. The pro-vascular strands and pith are completely differentiated and are narrowly hemispherical in outline. Through the latter part of June and through July the development continues, and reaches its climax late in July (Figs. 11 and 20a). By that time the average crown width index is about 140. Some crowns may be very broad, attaining a width index of 181. It is still convex in outline, though often flatter than it was during June and early July. The pro-meristematic tissue has become very shallow, often being reduced to a depth of 80 microns. The bases of the young leaves and scales approach the same level as the pro-meristematic tissue. Both the pro-vascular strands and the pith are broadly hemispherical in outline.

PHASE 3. Development of the Acutely Conical Internal Structure of the Winter Bud

From late July of year II ("on") to the winter of years II ("on") and III ("off"). Figs. 12 and 20b.

After the last of July, the tip continues to grow, but the growth between this time and winter produces a structure which is different from that found at the tip during any other phase of its development. To understand this

structure it must be remembered that by the first of August, when this new phase starts, the scales of the winter bud are almost mature and quite rigid. Growth then is limited to the internal or central portions of the bud. During this phase of development the surface of the crown rises well above the bases of the outer and larger bud scales, the width of the crown gradually narrows, its outline becomes less convex and the depth of the pro-meristematic tissue increases. The outlines of the pro-vascular strands and pith change from hemispherical to conical. An early stage may be seen by the middle of September. At this time the internal and active portions of the bud taken as a whole form a structure like a pyramid or broad-based cone. The climax of this phase is reached by November (Figs. 12 and 20b), and then the structure of the tip remains unchanged in its characteristic features throughout the winter. During this time (the winter of years II and III) the crown is so narrow that it may have a width index of only 73. A young primordium is always present. Its base is level with the upper surface of the crown. This upper surface of the crown is flat and at its periphery it slopes abruptly down to the base of the second youngest primordium. The base of this second youngest primordium is decidedly below the base of the youngest primordium and the surface of the crown. The pro-meristematic tissue may be 145 μ in depth, which is almost double that of any other stage. The pro-vascular strands and pith are acutely conical. All the internal portions of the bud now form a structure like a long, narrow or acute cone, with the crown and youngest primordium at the apex and the other primordia and young leaves arranged in a spiral down the steeply sloping sides. It is difficult to interpret the significance of all these features. The flat crown suggests inhibition of growth, but the increased vertical distance between the bases of the primordia, and the great increase in depth of the pro-meristematic tissue, indicate that some growth is taking place. The great depth of the pro-meristematic tissue suggests also that either differentiation is not taking place or is taking place very slowly. Certain median longitudinal sections of the tip at this stage, if taken by themselves, may lead to a false conception of tip structure. Should the section not include the youngest primordium, the crown will appear as a narrow, flat-topped, raised plateau. The whole appearance of the internal structure during this period gives the impression that the tip is growing within a restricted space and that the only outlet for its increasing length is to push up a narrow conical growth between the more or less rigid and inactive bud scales. Sections of buds from the December, January, February, and March collections suggest that a very slow growth and slight increase in size continues even during these months, but as yet this cannot be stated with certainty. In its characteristic features the form which the tip assumed by November, year II, remains almost unchanged until about the middle of March, year III.

PHASE 4. Rapid Elongation During the Early Spring of Year III

From early spring of year III ("off") to foliation during the last week of May, year III ("off"). Fig. 13.

Growth activity starts within the bud very early in the spring (year III,

"off"). Actual cell division is observed early in April. This is before there is any great change in the shape or size of the bud. During April and May the growth of the axis inside the bud is very rapid and produces a structure which is unlike that of any other stage (Fig. 13). The unique feature of this phase is due to a rapid growth in length which is not accompanied by a corresponding increase in diameter, hence the axis is pushed out into a long narrow cylinder. The crown becomes slightly broader and regains its convex outline. It proliferates young leaves and scales so rapidly that there is no great vertical distance between the bases of the two or three youngest primordia, but, due to the very rapid elongation of the axis just below the tip, the bases of the slightly older primordia and young leaves become very widely separated vertically. The usually fairly sharp boundary between embryonic cells and young pith cells is entirely lost and there is a long region in which the structure of the tissue is halfway between pro-meristem and pith. The strictly pro-meristematic tissue is not as deep as it was during the winter. Both the pro-vascular tissue and the pith are narrowly cylindrical. During this phase the long distance between the bases of the young leaves, their conspicuous and curved leaf traces, the parallel sides of the long narrow pro-vascular cylinder, and the long transitional region between pro-meristem and pith give to the median longitudinal section a decidedly unique and characteristic appearance.

PHASE 5. Broadening and Flattening of the Crown Previous to Flower Formation

From foliation during the last week of May, year III ("off"), to the latter part of July and first week of August, year III ("off"). Figs. 14 and 22a.

About the end of May the new and attenuated axis starts to broaden throughout its whole length. This is just about the time of full bloom for the "on" spur and the maturing and expanding of the leaves for all spurs. Early in June this broadening of the axis continues and is accompanied by decided and very rapid changes at the tip. Of these changes, the most conspicuous is in the width of the crown. It becomes very broad, attaining an average width index of 165, but some may reach a width index of 250. The contour of the crown is much less convex. Sometimes it is almost flat. The pro-meristematic tissue becomes very shallow, often having a depth of not more than 70μ. Most of the bases of the leaf primordia are level with the pro-meristematic region, giving the whole tip a "broad-shouldered" appearance in longitudinal section. The pro-vascular strands and pith are both broadly hemispherical in outline. The climax of this phase of development is reached during the first weeks of July, year III (Figs. 14 and 22a). At this stage it resembles the tip of the last of July, year II, except that it may be a little flatter, but it is decidedly broader and flatter than the tip of the same date (first week of July) year II. Wording this another way: by the first of July, the growing tip of the "off" spur is broader and flatter than the growing tip of the axillary branch of the "on" spur, but it is similar to the shape that the

tip of the axillary branch of the "on" spur will assume by the last of July. The tip that has just been described for early July, year III ("off"), with its broad flat crown, its shallow pro-meristematic tissue, its leaf primordium bases level with the pro-meristem and both pro-vascular strands and pith broadly hemispherical, is the structure that immediately precedes the initiation of flower primordia. It is found on the "off" spur during the first three weeks of July, year III.

PHASE 6. *Initiation of Flower Primordia*

> During the last two weeks of July or the first week of August, year III ("off").

Sometime during the last two weeks of July or the first week of August the formation of leaf primordia ceases and the five sepal primordia of a terminal flower are formed instead. In the proliferation of leaf primordia, during purely vegetative growth, the last-formed primordium always becomes elevated above those formed previously. During the development of the five sepal primordia, this elevation does not occur, consequently they all appear at the same level; that is, in the same horizontal plane. The result is, the shape of the crown as seen in transverse section changes from triangular to circular. This change in the shape of the upper surface of the crown and the appearance of the five sepal primordia are the first morphological indications of the initiation of flower formation.
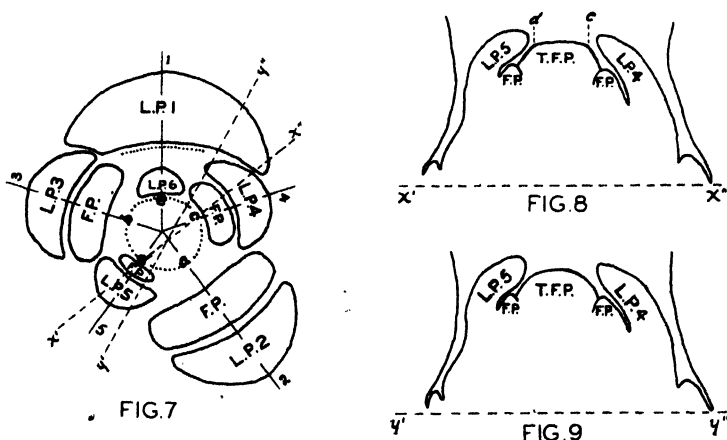
FIGS. 7 – 9. FIG. 7. *Diagrammatic horizontal projection of a flower-forming growing tip; L.P., leaf primordium; F.P., flower primordium; a, b, c, d, and e, points at which sepal primordia are appearing.* FIGS. 8 AND 9. *Enlarged longitudinal sections through x'–x'' and y'–y'' (Fig. 7) respectively; L.P., leaf primordium; F.P., flower primordium; T.F.P., terminal flower primordium; c and d, sepal primordia.*

The change from a triangular to a circular crown occurs very rapidly. Considering leaf primordium No. 6, Fig. 7, as the last leaf primordium formed, the five sepal primordia will appear at points *a, b, c, d,* and *e* in a circle at the periphery of the crown. The one at *a* appears first, then, in rapid succession,

b and *c*.   About the same time as *a* and *b* appear, but before the appearance of *c*, the tissue towards the periphery of the crown rises slightly.   This rise occurs between *a* and *b* and also beyond *b* towards the place where *c* will appear.   This raised margin, as seen in cross section, is not straight but is curved.   Thus, at this stage the margin of the crown from *a* to, and beyond, *b* forms a semicircle.   From *c* to *a* the margin is still practically a straight line. Primordium *c* then appears and the elevated circular margin is continued to *a*. Almost immediately, primordia *d* and *e* appear.   At this initial stage, primordium *a* is the largest and *e* the smallest.   Their gradation in size is represented by the alphabetical order.   The crown is now circular in outline (dotted circle, Fig. 7).   The five sepal primordia, and the slightly raised mass of pro-
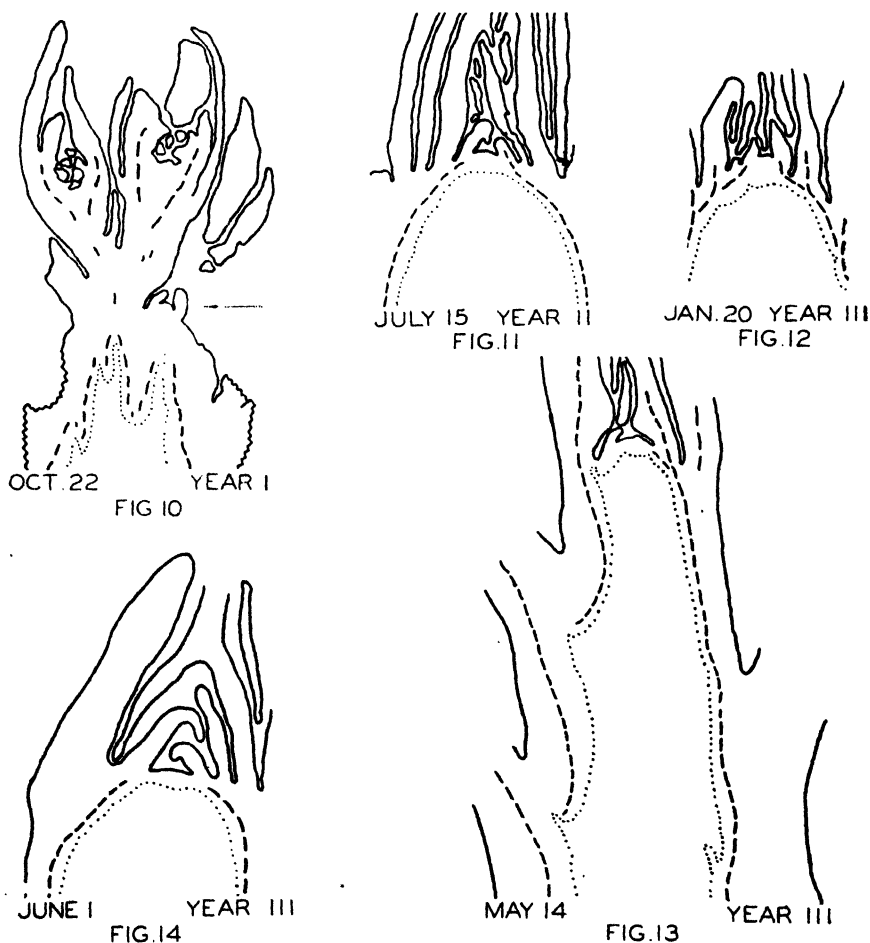
FIGS. 10 – 14.   *Camera lucida drawings of the median longitudinal section through the growing tip.   Date of collection is indicated on each figure.   Pro-vascular strands indicated by dashes; outline of pith indicated by dots.*   FIG. 10.   *Arrow points to primordium of new axillary branch.   Magnification 23.*

meristematic tissue from which these sepal primordia originate, form the primordium of the terminal flower.

In a longitudinal section of the tip at this stage the crown is seen to be either very slightly convex or quite flat. As the whole crown is raised without very much broadening of its base, the sides slope very steeply. In a longitudinal section through the sepal primordia (along line x'–x'', Fig. 7) the upper surface of the crown curves abruptly through an angle of about 45° to the steeply sloping surface of the side (Fig. 8). In sections between the sepal primordia (along line y'–y'', Fig. 7) the upper surface curves more gradually to the side (Fig. 9). These sepal primordia are not conspicuous in longitudinal section because, in the very early stages, they are not raised above the centre of the crown but merely form undulations at its periphery.

To observe the various steps in the appearance of the sepal primordia it is necessary to examine stained dissected buds under a compound microscope, and while the dissected bud is being examined in the ray of very strong light it must be rotated through 360°, otherwise shadows may give to the crown an appearance which is quite misleading. A good dissecting microscope is not suitable for these observations because its depth of focus is too great. Very slight elevations are not apparent, and the first appearance of the sepal primordia will not be observed. With the compound microscope and a sufficiently high magnification, the tips of the sepal primordia may be brought into focus, with the tissue between them out of focus. This tissue between the embryonic sepals will come into focus if the lenses are lowered very slightly. By this method minute elevations on the surface of the crown may be detected and the five sepal primordia at the periphery of the crown may be identified at a very early stage.

While the central and terminal flower is being differentiated, the primordia of the lateral flowers appear. These lateral flowers are all axillary and were observed about the same time as the appearance of primordium c, that is, while the crown was becoming circular. At first these axillary primordia are linear, tangentially elongated elevations of pro-meristematic tissue and appear in the axils of leaf primordia Nos. 2, 3, 4, and 5. The very early stages of their development provide the corrugations seen in longitudinal section by Goff (8). Longitudinal sections through x'–x'' or y'–y'', Fig. 7, would show a condition and are illustrated in Figs. 8 and 9. The flower primordia in the axils of the leaf primordia are pressed tightly against the terminal flower primordium and as they are all pro-meristematic tissue the terminal and lateral primordia appear, in longitudinal section, to have had a common origin, namely, the crown. But in the dissected buds the lateral flower primordia are seen to be separate and axillary structures (Fig. 7). The flower primordium in the axil of leaf primordium No. 2 may be recognized first, and in the early stages it is the largest of these lateral flower primordia. The others grade in size to that in the axil of leaf primordium No. 5, which is sometimes difficult to recognize in the very early stages. Narrow elongated zones of pro-meristematic tissue were recognized in the axils of leaf primordia

Nos. 6 and 1, but in the specimens examined these had not developed into flower primordia.

About the same time as the appearance of the sepal primordia *d* and *e* (Fig. 7), and when the axillary flower primordia are first apparent, all the flower-forming tissue becomes elevated very rapidly (Figs. 8, 9). The primordium of the terminal flower is always above the others. Its upper surface becomes slightly concave or saucer-shaped, but it is always circular as seen in cross section. The pro-vascular strands and pith follow into the elongating tip and become acutely conical in outline as seen in longitudinal section. This is probably the stage described by Bradford (4) and Magness (17) as the first indication of flower formation, and as the distal portion of the pro-meristemic tissue is not yet differentiated, this may also be the stage described by Kraus (15) as a "thickening of the axis", for the undifferentiated embryonic tissue could be considered as "thickened" whether it be measured horizontally or vertically. Finally, after further elevation, the terminal flower primordium becomes distinctly raised and develops a decidedly concave upper surface. The flower primordia in the axils of leaf primordia Nos. 2, 3, 4, and 5 develop into laterally compressed flower primordia. From the standpoint of gross morphology, the whole terminal structure is now established as an embryonic flower cluster.

From the description given above it is seen that the development of the terminal flower primordium is initiated slightly in advance of the others, but later proceeds concurrently with the development of the lateral flower primordia. In both, all stages follow each other rapidly, and only by dissecting a large number of buds, during the critical period, may all be seen.

It is evident that on the appearance of sepal primordium *e* (Fig. 7), the crown has ceased to exist as an apical organ of vegetative growth, and all its tissue has become differentiated into the terminal flower primordium. There is no question of there being any axillary pro-meristematic tissue in the structure of this terminal flower primordium, for after it is completely formed the axillary embryonic tissue may still be recognized in the axil of leaf primordium No. 6. Also, the terminal flower primordium is differentiated before the lateral flower primordia. This is in agreement with the findings reported by Black (3, p. 527) but is not in agreement with the statement made by Bradford (4, p. 5) that "The apical protuberance is differentiated last . . . .". The findings reported in the present investigation suggest that the inflorescence of the apple is "determinate", but that the order of development for the other flowers of the cluster, namely, those in the axils of leaf primordia Nos. 2, 3, 4, and 5, Fig. 7, is acropetal and not basipetal.

The time at which this development occurred varied with the variety. During one season with the McIntosh, these stages appeared by the third week of July; in a different season, with the Gravenstein, they occurred during the fourth week of July, and with the Wagener during the first week of August. However, for any one variety in any one season, differentiation was started and completed within a very short period, not more than two weeks. After

this period, during which the embryonic flower cluster stage was reached, no new initial stages of flower formation were observed.

Later Stages of Development

Later development includes completion of flower formation (Fig. 22b) expansion at blooming (Fig. 23), the setting, maturing, and ripening of the fruit (Fig. 24). All these stages have been excellently described by many investigators, as Drinkard (6), Kraus (15), Bradford (4), etc. Thus there is no need to repeat the description in this article.

The complete story for one growing tip is represented diagrammatically by Figs. 18 to 24. These should be of assistance in associating the steps in tip development, as seen under the microscope, with the familiar external appearance of the spur at each of the various stages. Crown width indices and measurements of depth of pro-meristematic tissue are given in Table II. In Figs. 15 and 16 the weekly averages from the two tables are plotted. A comparison of the two graphs suggests that on the average a broad crown is associated with the shallow pro-meristematic tissue and a narrow crown with deep pro-meristematic tissue.

Discussion

Some statements have already been made in explanation of the stages that former investigators have reported as the first indication of flower formation. These investigators do not report the shape of the crown as seen in transverse section changing from triangular to circular, nor do they correlate this with the first appearance of the sepal primordia, but Goff (8) described the appearance of this stage as seen in longitudinal section. The broad crown taken as the first indication of flower formation by Kirby (14), Tufts and Morrow (22), and Aaron (1), and the flat crown used by Gibbs and Swarbrick (7), are not sufficient, for about the last of July the purely vegetative tip will also have a broad and fairly flat crown. The thick and non-serial sections used by Rasmussen (21) would not be sufficient for an accurate determination of the earliest stages.

It can be stated as a general principle that many methods and many types of section are needed before one can make an accurate identification of the first morphological indication of flower formation.

As already stated, the period during which this initiation of flowers occurs is not extensive and does not last until late in the summer. However, there must be some real foundation for the many statements in the literature that the period is of long duration and may extend into the autumn. There are two probable explanations. First, in cases of abnormal development (and these are very common in plants) a structure may appear completely out of season. Some of the very late instances of flower initiation were probably of this nature. The second explanation is that occasionally some vegetative buds on the "on" tree have, during August, very broad crowns. When these become elevated, preparatory to the formation of the conical winter structure,
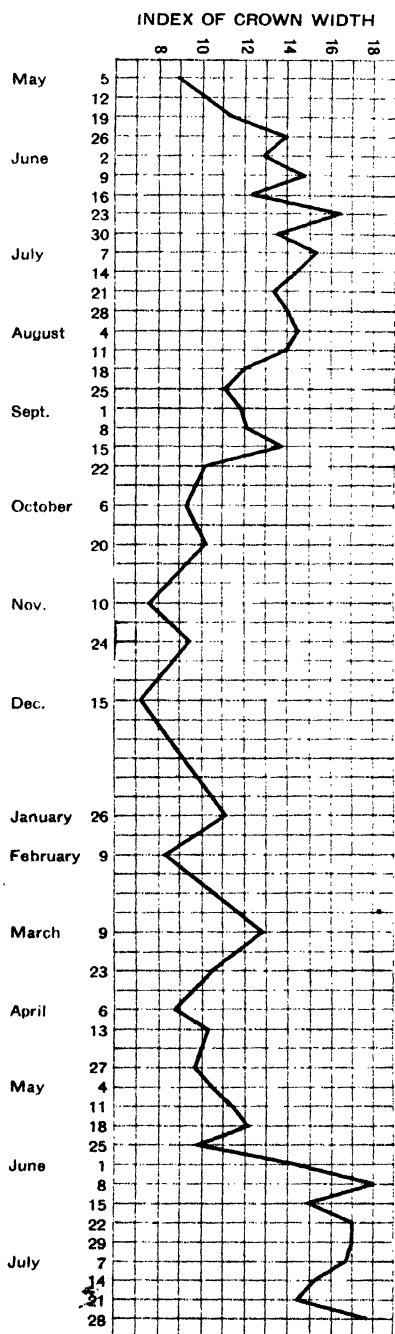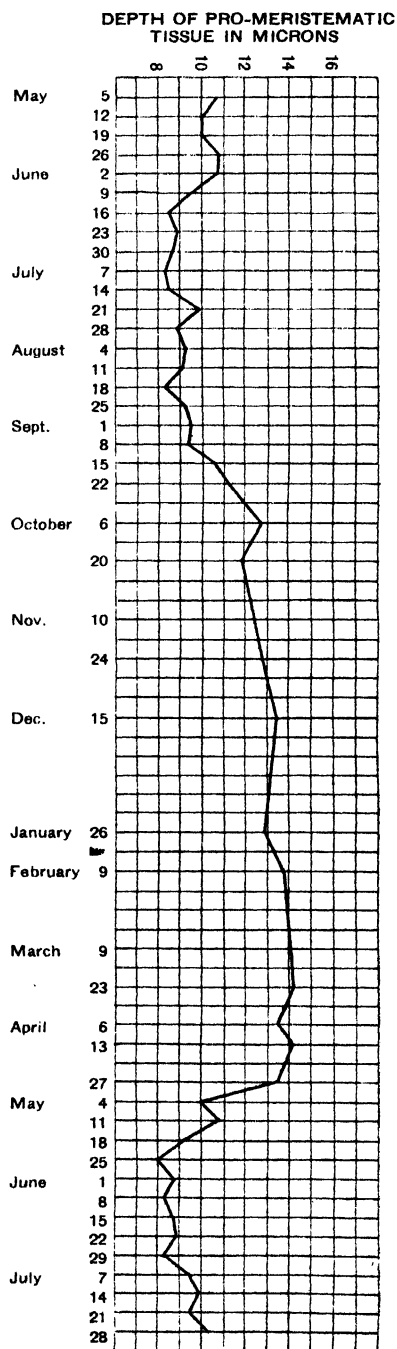
FIGS. 15 AND 16. FIG. 15. *Crown width index, plotted on a basis of the weekly average.*
FIG. 16. *Depth of pro-meristematic tissue, measured in microns and plotted on a basis of the weekly average.*

FIG.17

FIG 18

YEAR I   AUTUMN

FIG.19

FIG. 20

SPRING   YEAR II   AUTUMN
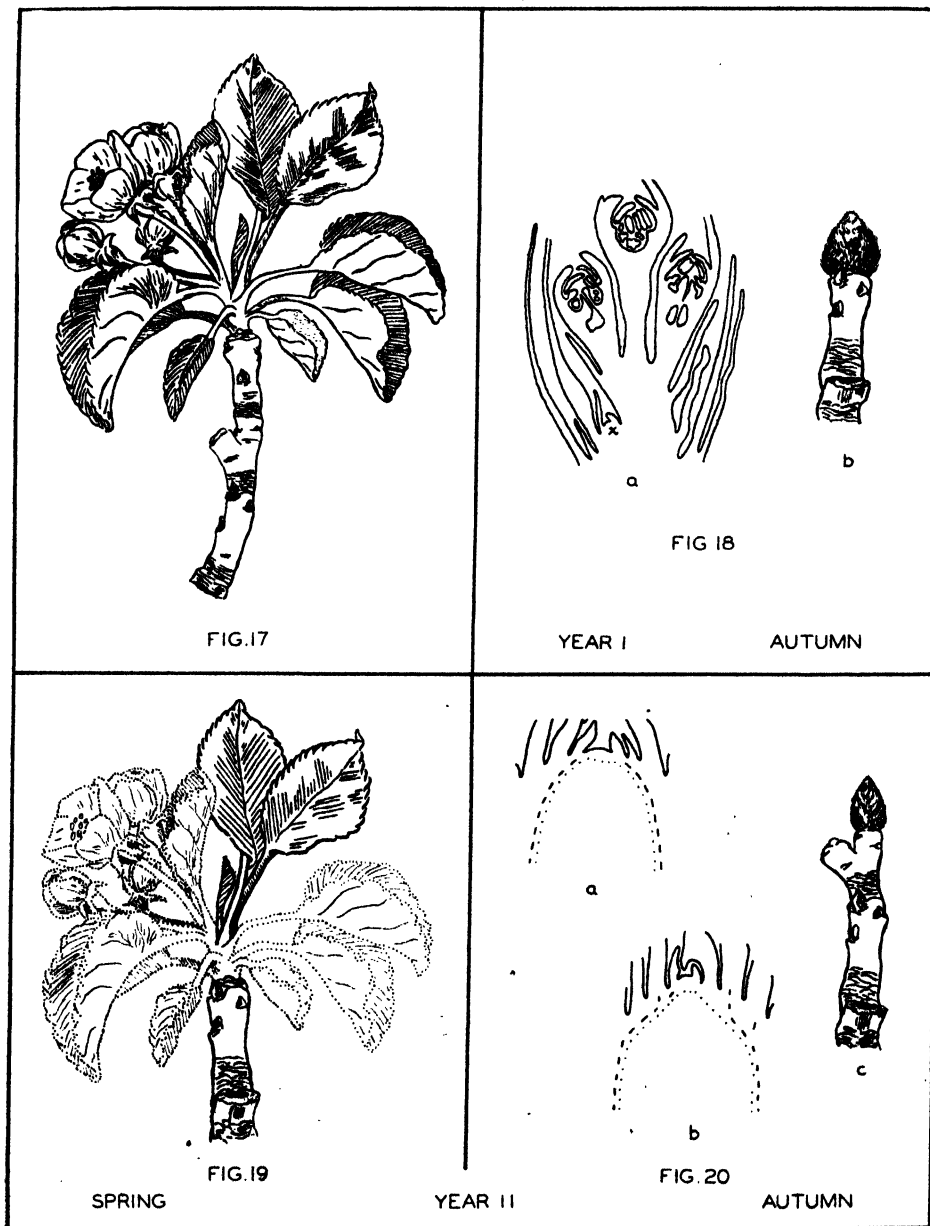
FIGS. 17 – 20.   FIG. 17.   *Typical biennially bearing fruit spur, illustrating flower cluster, young lateral branch and annual growth in length of spur.*   (FIGS. 18 – 24.   *The development of a single spur from late autumn year I, to harvest time, autumn year IV.*)   FIG. 18.   *Winter bud (mixed) autumn year I "off".   a, longitudinal section; x, new axillary growth; b, habit sketch of bud and spur.*   FIG. 19.   *Spur at blossom time, spring year II "on".   Flower cluster and its leaves stippled, new lateral shoot lined.*   FIG. 20.   *Development of new lateral shoot, year II "on".   a, longitudinal section of mid-summer tip; b and c, late autumn; b, longitudinal section of tip; c, habit sketch of winter bud (leaf) and spur.*

FIG.21

SPRING

FIG.22

YEAR III

AUTUMN

FIG 23

SPRING

FIG.24
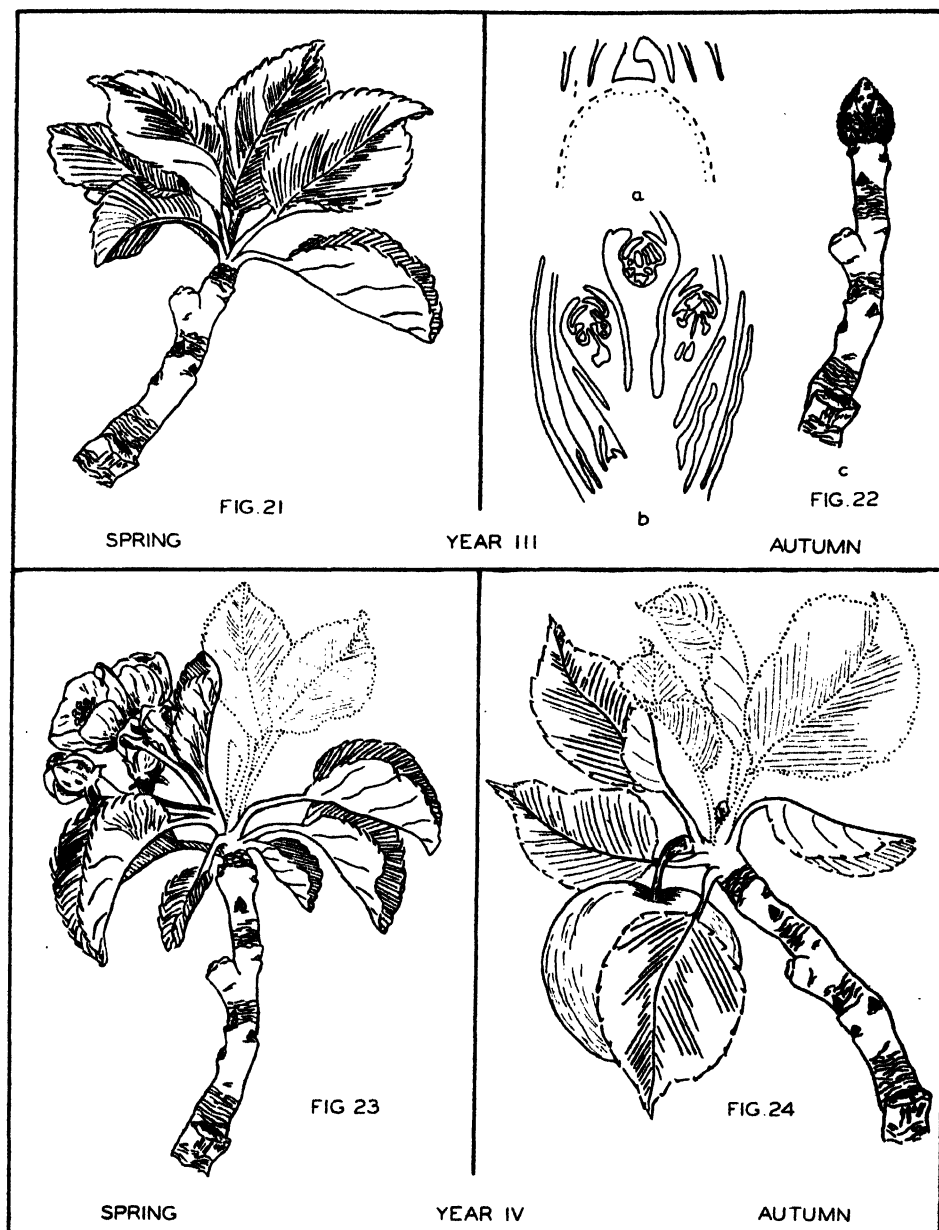
YEAR IV

AUTUMN

FIGS. 21 – 24. FIG. 21. *Spur at time of foliation, spring, year III "off".* FIG. 22. *Flower bud and spur, year III "off".* a, *tip at end of June or first of July, i.e., just prior to flower formation;* b *and* c, *autumn;* b, *longitudinal section of bud;* c, *habit of sketch of bud and spur.* FIG. 23. *Spur at blossom time, spring, year IV "on".* *Flower cluster and its leaves lined, new lateral shoot stippled.* FIG. 24. *Spur at harvest time, autumn, year IV "on".* *Fruit and the leaves that expanded with flower cluster, lined; new lateral shoot and its leaves, stippled.*

their appearance in longitudinal section is similar to that of the potential flower bud at the commencement of flower formation when elevation is first apparent. It is possible that the late summer stage of these vegetative buds may have been mistaken for the early stage of flower initiation.

Conclusion

In conclusion, reference should be made to the original purpose of this investigation, namely, the first morphological indication of flower differentiation. This might have reference to merely the first appearance of a structure which will develop into a part of the flower, or to a change in structure which, though not a stage in flower formation, could be taken as an indication that physiological differentiation of a fruiting tip is in progress. In the first and purely morphological aspect the purpose of the investigation has been fulfilled. The first morphological indications of flower differentiation were found to be the change in shape of the upper surface of the crown, as seen in transverse section, from triangular to circular and the appearance of the five sepal primordia, and this takes place during the last two weeks of July or the first week of August. This is of interest from the standpoint of morphology, but, to one wishing to influence flower differentiation, it is of little interest, for physiological differentiation must precede morphological differentiation. The crown becoming circular indicates that the question of flower differentiation is settled, and probably cannot be influenced very much one way or the other. Thus, for those who wish to influence flower initiation, it is much more important to discover a morphological change that would indicate that physiological differentiation is in progress. The detailed account of tip development given above may be some help in the discovery of this. The authors wish to make a suggestion. The major morphological changes that immediately precede the initiation of flower primordia are those described in Phase 5, and commence in the "off" bud during the first two weeks of June or just after foliation. In the vegetative bud a similar change does not occur until over a month later. It is suggested that this early broadening and flattening of the crown in the "off" bud during the early part of June may be a morphological indication that physiological differentiation is taking place. Of course, this is only a suggestion, for, before it can be proposed even as a theory, experimentation is necessary.

Acknowledgments

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VARIETAL DIFFERENCES IN BARLEYS AND MALTS

VII. STARCH-LIQUEFYING ACTIVITY, AUTOLYTIC DIASTATIC ACTIVITY AND THEIR CORRELATIONS WITH SACCHARIFYING AND PROTEOLYTIC ACTIVITY[1]

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Abstract

Determinations made on 144 samples of malt, representing 12 varieties grown at 12 experimental stations in Canada, show that varietal differences exist with respect to starch liquefying activity (max. 768, min. 275 units) and autolytic diastatic activity (max. 958, min. 664 units). Varieties of poor malting quality tend to be low with respect to both properties. The effect of environment is also considerable (liquefying, max. 510, min. 288; autolytic, max. 806, min. 704).

The correlation coefficients among liquefying, autolytic diastatic, saccharifying, and proteolytic activities of malt and total barley saccharifying activity were studied. Significant *inter-varietal* associations exist between each pair of properties, but partial correlation studies suggest that only those between saccharifying activities of barley and malt ($r = 0.90$), and between liquefying and autolytic activities of malt ($r = 0.97$), represent real and close relations. The other associations between pairs of enzymatic activities seem to reflect mainly positive correlations between each activity and total salt-soluble nitrogen in the barleys.

Significant *intra-varietal* associations exist between each pair of enzymatic activities, and between each activity and total barley nitrogen. It appears that environmental factors which tend to increase total nitrogen also tend to increase each enzymatic activity, but these do not increase regularly with respect to each other and are not closely related. Partial correlations independent of total nitrogen suggest that only barley and malt saccharifying activities ($r = 0.67$) and liquefying and autolytic activities of malt ($r = 0.63$) are related within varieties.

It appears that the rate of autolysis in samples of different varieties from the same station is controlled almost entirely by starch liquefying activity, but the latter property is not the limiting factor controlling autolysis in samples of the same variety from different stations. Within varieties some other factor, presumably starch resistance, must play an important part.

Lintner values determined on 144 samples of barley and on the malts made from them were reported in Part II (11) of this series, together with the results of a statistical examination of the relations between these properties. Data on the proteolytic activity of the same malts were given in Part VI (4). Further investigations of the enzymatic activities of these malts, namely,

determinations of starch-liquefying activity and of autolytic diastatic activity, are reported in the present paper.

It is generally agreed that the saccharifying activities of barley (total by the papain method, 11) and of malt measure β-amylase activity. In malt β-amylase doubtless contributes most of the effect, but α-amylase also plays a part in saccharification. The activity of the α-amylase in malt has commonly been measured by methods involving changes in the starch-iodine colour (*e.g.*, 14), but Blom, Bak, and Braae (7) consider that starch-liquefying activity is a better method for estimating this enzyme. They point out, however, that β-amylase has some slight effect on starch-liquefying activity, but conclude that for all practical purposes the liquefying activity can be considered as a measure of α-amylase activity. Considerable doubt has been cast on the validity of the usual starch iodine methods by the recent work of Hanes and Cattle (8). They have shown that the rate of colour change produced by α-amylase is considerably increased in the presence of β-amylase. It, therefore, appears that starch-liquefying methods give a more reliable measure of α-amylase activity than methods based on starch iodine coloration.

It does not yet seem possible to enumerate the factors controlling the autolytic diastatic activity of malt as measured by methods of the Blish-Sandstedt type (5). On *a priori* grounds, it might be assumed that the rate of autolysis is dependent on factors such as starch-liquefying activity, saccharifying activity, and the resistance of the substrate to enzymatic attack. Shellenberger and Bailey (13), working on these assumptions, were unable to demonstrate any close relations among these properties and autolytic diastatic activity.

Blish *et al.* (6, 12) have studied autolytic saccharification in flour and the effect of adding malt extract to the digests. They were concerned mainly with the factors influencing the final degree of saccharification, rather than the rate, and their work bears rather indirectly on the problem under discussion. Further investigation seemed called for and it was mainly for this reason that the work reported in this paper was undertaken.

Since the data were available, a study of the relations among enzymatic activities, *i.e.*, saccharifying (11), proteolytic (4), autolytic diastatic, and starch-liquefying, is given in the present paper.

Materials

The malts used in the study are those used in previous studies in this series (1–4, 10, 11). They represent 12 varieties of barley (listed in Table I), grown at 12 widely separated experimental stations in Canada (listed in Table II). The barley varieties and the methods used in growing the samples were described in detail in Part I (1) of this series and the malting methods and commonly measured properties of the malt, including Lintner values, were reported in Part IV (10).

Methods

Starch-liquefying Activity

The method described by Jozsa and Johnston (9) was used for the determination of starch-liquefying activity. The following modifications were made to facilitate manipulation. Infusions of the malts were made, using 500 ml. of 5% sodium chloride instead of 1 l. of 2.5% solution, and this extract was diluted 1 to 20 with water. Enzymatic digests were made in 250-ml. wide-mouthed Erlenmeyer flasks.

The 100-ml. pipette used to determine viscosity was calibrated by the method of Jozsa and Johnston and it was found that their conversion tables could be used to convert the results to "liquefons" per gram of malt. These authors define a liquefon as "that amount of starch-liquefying enzyme which will convert the standard starch paste at the rate of 25 mg. of dry starch per minute at zero time under the specified conditions."

Autolytic Diastatic Activity

The following modification of the Blish-Sandstedt (5) method was used.

Two 5-gm. aliquots of the finely ground malt were weighed into 100-ml. glass stoppered Florence flasks and subjected to autodigestion at 35° C. Digestion of one aliquot was stopped at the end of one hour in the usual manner and digestion of the second aliquot after two hours. After filtering the digests, 1 ml. of the filtrate was used for a determination of reducing compounds by a modification of the ferricyanide method, which permitted the estimation of larger quantities of sugar. The ferricyanide reagent used was calibrated against a solution of pure maltose, and autolytic activity was expressed as milligrams of maltose produced from 10 gm. of malt during the second hour of digestion.

Typical curves showing the relation between reducing compounds produced and time of digestion for samples of two varieties of barley are shown in Fig. 1. The upper and lower curves represent samples of O.A.C. 21 and Wisconsin 38 grown at the same station. A curvilinear relation exists between 0 and 1 hr., whereas the relation between 1 and 2 hr. is almost linear. Other experiments have shown that if digestion is carried much beyond 2.5 hr. a definite drop in the rate of production of reducing compounds occurs. It seems probable that during the early stages of digestion the fractured starch grains are subjected to ready attack with the result that the initial rate of production of reducing compounds is greatest. When the readily attacked starch has been utilized, the rate of digestion becomes relatively constant until it is retarded by reduced substrate concentration and by the inhibiting effects of hydrolytic products. We have attempted to measure the rate during the period when it is relatively constant.

The dotted curve in Fig. 1 represents the same sample of O.A.C. 21 ground less finely. It will be observed that the two curves for O.A.C. 21 are very similar and it is evident that the method is to some extent independent of the fineness of grinding. This is an important advantage in dealing with samples

grown under widely different environmental conditions and thus differing considerably in their resistance to grinding.
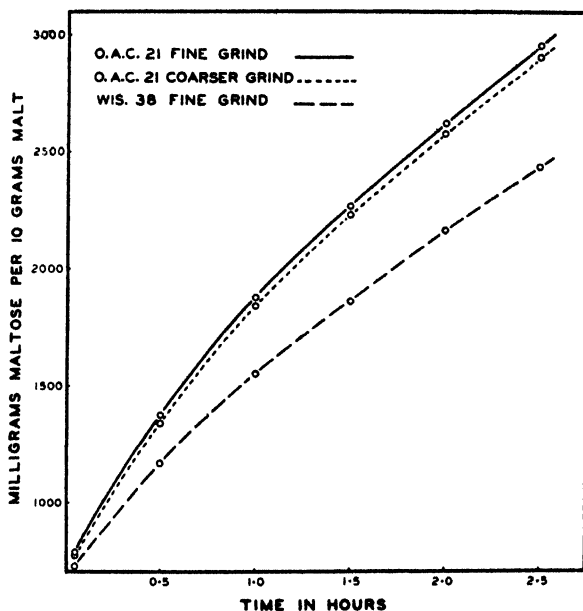
FIG. 1. *The relation between reducing substances produced and time of autodigestion. Values represent means of duplicate determinations.*

Replication

Duplicate determinations were made on one-third of the samples to provide a check on the precision of the determinations. These replicates were selected at random after imposing the limitation that four samples of each of the 12 varieties and four samples from each of the 12 stations should be selected. The standard deviations of single determinations were: Autolytic activity, mean = 765, S.D. = 20.4 or 2.7% of the mean; starch-liquefying activity, mean = 410, S.D. = 11.0 or 2.7% of the mean.

Mean Differences Between Varieties and Between Stations

The results of the investigation of autolytic activity and starch-liquefying activity are summarized in Table I as means for each variety, over all stations, and in Table II as means for each station, over all varieties. The tables also contain, for purposes of comparison, the corresponding mean values for saccharifying activity which were previously reported in Part II (11).

Owing to the differential effect of environment on varieties, these did not fall in the same order at all stations, nor did the stations fall in the same order with respect to each variety. An analysis of variance was necessary to determine whether differences between varietal means, and between station means, could be considered significant. The results of the analyses are put on record

in Table III, but for the purposes of the following discussion they are summarized (last lines of Table I and II) as necessary differences between means required for a 5% level of significance.

TABLE I

VARIETAL MEANS FOR AUTOLYTIC DIASTATIC ACTIVITY, STARCH LIQUEFYING ACTIVITY
AND SACCHARIFYING ACTIVITY OF MALT

Class	Variety	Autolytic diastatic activity, mg. maltose/10 gm.	Starch liquefying activity, liquefons/gm.	Saccharifying activity, °Lintner
Six-rowed, rough-awned	Olli	958	768	153
	O.A.C. 21	785	419	127
	Peatland	779	444	120
	Mensury	777	406	129
	Pontiac	770	412	131
Six-rowed, smooth-awned	Velvet	764	385	124
	Nobarb	678	281	100
	Regal	676	275	85
	Wisconsin 38	664	281	96
Two-rowed, rough-awned	Hannchen	804	418	115
	Victory	787	435	103
	Charlottetown 80	739	392	100
Necessary difference, 5% level		57	54	11

Varietal Differences

The data given in Table I show conclusively that varietal differences exist with respect to each of the three properties measured.   Olli gives consistently high values, whereas those for Regal and Wisconsin 38 are consistently low. There is little indication of differences between the various classes of barleys since, although the lowest values are given by three of the smooth-awned varieties, the fourth, Velvet, gives values of about the same order as the rough-awned varieties.   In Canada, O.A.C. 21, Mensury, and Olli are considered good malting varieties, whereas Nobarb, Regal, and Wisconsin are considered poor.   The data for these varieties thus suggest that good malting varieties are characterized by reasonably high diastatic activities.

Station Differences

The mean values for each station are given in Table II.   Comparison of the differences between these with the necessary difference required for a 5% level of significance leaves no room for doubt that environment has a considerable effect on all three properties.   It is interesting to note that the maximum spreads between station means, 102 for autolytic activity and 222 for liquefying activity, are considerably lower than the corresponding spreads between varietal means, 294 and 493.   Since the stations cover a wide range of environment, from a dry continental climate to a moist maritime one, it

appears that variety has a far greater effect on autolytic diastatic activity and starch-liquefying activity than environment. It is also worth noting that most of the variation between station means, with respect to autolytic activity, is caused by four stations, the first two and last two in the test. Values for the remaining eight stations are closely grouped and do not differ significantly.

TABLE II

STATION MEANS FOR AUTOLYTIC DIASTATIC ACTIVITY, STARCH LIQUEFYING ACTIVITY AND SACCHARIFYING ACTIVITY OF MALT

Station	Autolytic diastatic activity, mg. maltose/10 gm.	Starch liquefying activity, liquefons/gm.	Saccharifying activity, °Lintner
Fredericton	806	375	85
Ste. Anne de Bellevue	802	393	100
Brandon	788	441	117
Winnipeg	781	428	105
Guelph	778	510	122
Beaverlodge	777	488	150
Ottawa	767	422	133
Gilbert Plains	762	382	133
Nappan	752	288	63
Ste. Anne de la Pocatière	741	424	121
Lacombe	726	418	139
Lethbridge	704	348	116
Necessary difference, 5% level	57	54	11

Analyses of Variance

The variances of the data for each determination were analyzed into portions resulting from (i) average differences between varieties; (ii) average differences between stations; and (iii) remainder. The last portion results not only from variations caused by a true interaction between stations and varieties, but also from variations caused by soil heterogeneity within stations, and by sampling and analytical errors. It, therefore, provides an adequate criterion for testing the significance of differences between station and varietal means.

·The mean squares obtained by the analyses of variance are reported in Table III. Since the mean squares resulting from differences in the average performance of individual varieties, and from differences in the average performance of all varieties at different stations, are significantly greater than the corresponding remainders, it is apparent that significant differences exist between varietal means and between station means.

.In previous papers of this series, results of analyses of variance of data on 19 properties of the same sets of barleys or malts have been reported. For most of these properties, the variance due to stations proved considerably greater than that due to varieties. It is thus a matter of some interest that the reverse is true with respect to starch-liquefying activity and autolytic diastatic activity. The comparatively greater influence of variety on these properties is thus demonstrated.

TABLE III

ANALYSIS OF VARIANCE FOR STARCH-LIQUEFYING ACTIVITY AND AUTOLYTIC DIASTATIC
ACTIVITY

Variance due to	Degrees of freedom	Mean square	
		Starch liquefying activity	Autolytic diastatic activity
Varieties	11	198,909**	7,268**
Stations	11	42,074**	1,098*
Remainder	121	4,337	490

NOTE: *In this and later tables* ** *denotes that the 1%, and* * *that the 5%, level of significance has been attained.*

Correlation Studies

In studying the relations between various barley and malt properties, it is advisable to examine the inter- and intra-varietal correlations separately. These may differ considerably since the former are controlled by genetic factors, whereas the latter are controlled by environmental factors. There is thus little reason for expecting that the inter- and intra-varietal correlations will be similar, and they rarely are. For this reason the two kinds of relations are discussed separately in the following sections.

Inter-varietal Relations

The inter-varietal correlation coefficients between each pair of enzymatic activities are given in Table IV. Since all of these are significant and all but two are highly significant, it is obvious that an inter-varietal association exists between the various activities so that varieties which tend to be high in one enzymatic activity also tend to be high in other enzymatic activities.

It was shown in previous papers of this series that inter-varietal relations existed between barley saccharifying activity, malt saccharifying activity (3), and proteolytic activity (4) on the one hand, and the more soluble barley nitrogen fractions (*e.g.*, non-protein nitrogen, salt-soluble protein nitrogen, and total salt-soluble nitrogen) on the other. Correlations between these

TABLE IV

INTER-VARIETAL SIMPLE CORRELATION COEFFICIENTS BETWEEN ENZYMATIC ACTIVITIES

Enzymatic activity	Malt			
	Sacchari-fying	Lique-fying	Autolytic diastatic	Proteo-lytic
Barley saccharifying	0.904**	0.750**	0.751**	0.634*
Malt saccharifying	—	.816**	.856**	.662*
Malt liquefying	—	—	.975**	.798**
Malt autolytic diastatic	—	—	—	.807**

activities and total nitrogen, insoluble nitrogen or alcohol-soluble nitrogen were not significant. Similar relations have now been found between starch-liquefying and autolytic diastatic activities and the various nitrogen fractions. The most striking feature of these studies is the existence of significant inter-varietal correlations between all the enzymatic properties and the more soluble nitrogen fractions of the barleys. The correlation coefficients for these activities and total salt-soluble nitrogen are shown in Table V.

TABLE V

INTER-VARIETAL CORRELATION COEFFICIENTS AMONG ENZYMATIC ACTIVITIES (x), TOTAL BARLEY NITROGEN (n) AND SALT-SOLUBLE BARLEY NITROGEN (s)

x = enzymatic activity	Correlation coefficient		
	Simple, r_{xn}	Simple, r_{xs}	Partial, r_{xs-n}
Barley saccharifying	0.199	0.739**	0.737**
Malt saccharifying	− .039	.727**	.759**
Malt liquefying	− .103	.727**	.788**
Malt autolytic diastatic	− .136	.750**	.814**
Malt proteolytic	.070	.871**	.882**

To elucidate these inter-varietal relations the regressions of the enzymatic activities on total salt-soluble nitrogen were calculated by stations and tested for homogeneity. This analysis is shown in Table VI. It was found that the regressions did not differ significantly between stations. But, since the individual regressions only account for about one-third of the total variance of the enzymatic activities, it is obvious that total salt-soluble nitrogen is not the most important factor controlling the development of these activities. Similar relations can be shown for the other soluble nitrogen fractions, namely, non-protein and salt-soluble protein nitrogen. It seems probable that these soluble nitrogen fractions reflect some more fundamental property of the barleys that controls the development of the enzymatic activities in the malts.

TABLE VI

TEST OF HOMOGENEITY OF INTER-VARIETAL REGRESSION COEFFICIENTS BY ANALYSIS OF RESIDUAL VARIANCE

Variance due to	Degrees of freedom	Mean square				
		Barley saccharifying	Malt saccharifying	Autolytic diastatic	Starch liquefying	Autolytic proteolytic
Differences among station regression coefficients	11	640.85	286.25	9,999.6	14,499	1,267.3
Deviations from individual station regressions	120	727.28	345.01	8,417.4	14,847	1,906.4
Percentage of variance accounted for by individual regressions		36.8	37.5	27.5	34.3	37.0

To determine whether there is any real relation between the pairs of enzymatic activities, apart from that due to a common association with salt-soluble nitrogen, partial correlation coefficients between each pair of activities and salt-soluble nitrogen were calculated. These partial correlation coefficients appear in Table VII. Those between proteolytic and saccharifying activities are of negligible magnitude and it is inferred that the simple correlation coefficients (Table IV) reflect the relations between each of these activities and salt-soluble barley nitrogen.

TABLE VII

INTER-VARIETAL PARTIAL CORRELATION COEFFICIENTS, BETWEEN ENZYMATIC ACTIVITIES, INDEPENDENT OF SALT-SOLUBLE BARLEY NITROGEN

Enzymatic activity	Malt			
	Saccharifying	Liquefying	Autolytic diastatic	Proteolytic
Barley saccharifying	0.780**	0.460	0.441	−0.030
Malt saccharifying	—	.610*	.684*	.087
Malt liquefying	—	—	.946**	.399
Malt autolytic diastatic	—	—	—	.437

The following partial correlation coefficients drop below the 5% level of significance: barley saccharifying-liquefying; barley saccharifying-autolytic; proteolytic-liquefying; and proteolytic-autolytic. The available data thus fail to demonstrate that relations exist between these pairs of activities, which are independent of the relations between each of them and salt-soluble nitrogen. Nevertheless, the correlation coefficients are high enough to suggest that loose associations may exist and might be demonstrated by a study of a larger number of varieties.

The relation between barley and malt saccharifying activities is quite close as indicated by the simple correlation coefficient of 0.904. The partial correlation coefficient independent of salt-soluble nitrogen, 0.780, is significant to the 1% level. Since malt saccharifying activity results from the activity of β-amylase already present in the barley, this result was to be expected. When regression of malt saccharifying on barley saccharifying activity was determined by stations, it was found that these regressions did not differ significantly from station to station and would account for 76% of the variance of malt saccharifying activity. Total barley saccharifying activity (papain) is therefore the most important single factor controlling the development of malt saccharifying activity. However, since the removal of those portions of the variance associated with salt-soluble nitrogen affects the relation, it appears that other factors are operating to some extent.

In Part II (11) it was suggested that barley saccharifying activities might be of value in predicting malt saccharifying activities of plant breeders' samples. It was also stated that the lack of complete correspondence between

the two activities might be due either to the saccharifying activity of α-amylase or to differences in the responses of varieties to the particular malting conditions used in the investigation. The latter seems more probable, since the multiple correlation coefficient between malt saccharifying activity and barley saccharifying and starch-liquefying activities, 0.928, is not significantly higher than the simple coefficient, 0.904, but the multiple correlation coefficient between malt saccharifying activity and barley saccharifying activity and index of nitrogen modification, 0.952, is significantly higher than the simple coefficient.

A significant degree of association persists between malt saccharifying and liquefying activities, independent of salt-soluble nitrogen. It thus appears that there may be a direct association between these two activities.

The inter-varietal relation between starch-liquefying and autolytic diastatic activities is very close ($r = 0.975$; partial, independent of salt-soluble nitrogen, $= 0.946$). Individual regression equations of autolytic on liquefying activities account for 81.5% of the variance due to autolytic activity. It therefore appears that the liquefying activity of α-amylase is a factor of primary importance governing the rate of autolysis of malts made from barleys grown at the same station.

The relative importance of the saccharifying and liquefying activities in controlling the rate of autolysis in samples of different varieties can be demonstrated by the following partial correlation coefficients.

Autolytic $\times$ liquefying, independent of saccharifying, 0.934**.

Autolytic $\times$ saccharifying, independent of liquefying, 0.466.

Since the first of these is highly significant, whereas the second is not significant, it is clear that liquefying activity is a major, and saccharifying activity a minor, factor governing the rate of autolysis. This is also shown by the fact that the multiple correlation coefficient for autolytic activity and liquefying and saccharifying activities, 0.981, is not significantly higher than the simple coefficient between autolytic and liquefying activities.

Intra-varietal Relations

It has been shown throughout these studies that within varieties almost all properties are correlated with total barley nitrogen. In these circumstances simple intra-varietal correlation coefficients are frequently misleading, since they tend to reflect correlations between nitrogen and the variables under consideration, rather than the true relations between the variables. For this reason the intra-varietal relations discussed in this section are represented in Table VIII by both simple correlation coefficients and partial correlation coefficients independent of total nitrogen.

In considering the simple correlation coefficients given in the upper part of Table VIII, it is simplest to note first that autolytic diastatic activity is not significantly correlated with any of the other properties. It appears that within varieties autolytic activity is controlled by a number of factors, which vary independently with change in environment. Among these factors lique-

fying and saccharifying activity are doubtless numbered, but the roles they play are not of sufficient importance to produce significant simple correlation coefficients.

TABLE VIII

INTRA-VARIETAL SIMPLE CORRELATION COEFFICIENTS BETWEEN ENZYMATIC ACTIVITIES AND TOTAL BARLEY NITROGEN, AND PARTIAL CORRELATION COEFFICIENTS BETWEEN ENZYMATIC ACTIVITIES INDEPENDENT OF TOTAL BARLEY NITROGEN

Enzymatic activity	Total barley nitrogen	Malt			
		Sacchari-fying	Lique-fying	Autolytic diastatic	Proteo-lytic
Simple correlation coefficients					
Barley saccharifying	0.976**	0.978**	0.589*	−0.302	0.841**
Malt saccharifying	.962**	—	.690*	− .234	.879**
Malt liquefying	.632*	—	—	.300	.728**
Malt autolytic diastatic	− .269	—	—	—	.026
Malt proteolytic	.854**	—	—	—	—
Partial correlation coefficients, independent of total nitrogen					
Barley saccharifying	—	.669*	− .162	− .189	.070
Malt saccharifying	—	—	.386	.095	.406
Malt liquefying	—	—	—	.630*	.467
Malt autolytic diastatic	—	—	—	—	.510

All other activities are correlated with total nitrogen and among themselves. It thus appears that a change in environment that increases total barley nitrogen also tends to increase each of the enzymatic activities under consideration. Among the inter-station correlation coefficients only that between barley and malt saccharifying activities is particularly high. It thus appears that these two activities are quite closely related within varieties as well as between varieties.

Among the partial correlations independent of total nitrogen, only two are significant. One of these represents the relation between barley and malt saccharifying activities.

The other significant partial correlation coefficient is that between autolytic activity and liquefying activity. It is apparent that some relation exists between these two activities within varieties (as well as between varieties) but that this relation is masked in the simple correlation by the complicating effect of correlations with total nitrogen. It is possible that within varieties total nitrogen is correlated with some factor that has a considerable influence on autolytic activity, for instance, substrate resistance. When the effects of differences in total nitrogen are removed by calculating the partial correlation coefficient, some of the effects of differences in substrate resistance are probably removed also. Under these conditions it then becomes possible to demonstrate the relation between starch-liquefying activity and rate of autolysis, i.e., the partial correlation coefficient is found to be significant.

In general, there is some degree of similarity between the inter- and intra-varietal correlations. In both instances a close relation exists between barley and malt saccharifying activities, starch-liquefying activity is shown to be an important enzymatic factor governing rate of autolysis, and rather loose associations exist between the other pairs of activities.

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PHYSIOLOGICAL ACTIVITY OF A SERIES OF INDOLYL ACIDS[1]

By N. H. Grace[2]

Abstract

The physiological activity of a series of indolyl acids, from the acetic to the valeric, including 5-methyl-indolylpropionic, has been determined by the rooting responses of *Lonicera tartarica* cuttings treated with solutions of each. Indolyl-butyric acid was the most active chemical, affecting the number and length of roots per rooted cutting, the mean root length, the green weight of leaves, and the fresh root weights. Indolylacetic acid had significant effects on the number and length of roots per rooted cutting. Slight activity was shown by indolylpropionic acid, but neither indolylvaleric acid nor 5-methyl-indolylpropionic showed any significant treatment effects. None of the acids affected the number of cuttings rooted.

A recent communication gives the results of experiments in which plant cuttings were treated with a series of naphthyl acids from 1-naphthyl-acetic to ε-(1-naphthyl)-hexoic (2). It was demonstrated that physiological activity was shown by all members of the series tested, and the greater activity of the acids with an even number of carbon atoms in the side chain was the most striking feature of the results. It was considered of interest to carry out a similar experiment with a series of indolyl acids. In consequence, *Lonicera tartarica* L. cuttings, as used previously, were treated with indolyl acids from indolyl-3-acetic to α(3-indolyl)-valeric acid, and with β-3(5-methyl-indolyl)-propionic acid.

Experimental

The details of statistical arrangement and treatment were identical with those previously described, excepting that four, not five, replicates of 10 cuttings were used in this experiment (2). As with the naphthyl acids, solutions were prepared in 100 p.p.m. of K_2HPO_4, and this concentration of phosphate was used on the controls and with each chemical at all three concentrations, namely: 10, 50, and 100 p.p.m.

Dormant cuttings* were approximately 12 in. long, and were treated in groups of 40 (the four replicates) in 150 cc. of solution for 24 hr.; 720 cuttings were required for the experiment. They were rinsed and immediately planted in random order in brown sand in a propagation frame equipped with bottom heat cables. Sand temperature was maintained at 72° F., while that of the room approximated 65° F. The present experiment received more light than the former, as the days were longer during the present experiment. Apart from light effects the series of treatments of *Lonicera tartarica* with naphthyl and indolyl acids were carried out under closely similar conditions. The

cuttings were planted March 9 and removed for counts and measurements April 19, 1939.

In addition to the counts and measurements made on *Lonicera* treated with naphthyl acids (number of cuttings rooted, number and length of roots per rooted cutting, and the mean root length), weight of leaves and fresh root weights were determined. All the data thus secured were subjected to analyses of variance.

Results

The number of cuttings rooted in this experiment achieved a rather high general level; over all, 65% of those planted formed roots, as contrasted with about 35% rooting for untreated controls in previous studies with this plant (3, 4). Apparently this general stimulation was due to a uniform treatment of nutrient phosphate, K_2HPO_4 ; nutrient treatments of *Lonicera* cuttings have been shown to stimulate rooting (5). The response of this collection of cuttings to nutrient treatment may have masked the effects of the hormone on the number rooted.

It will be observed from Table I that there were significant differences due to the use of chemicals in respect of the remaining five characters. No significant interaction could be demonstrated; *i.e.*, there was no differential response at different dosages to the same chemical.

TABLE I

ANALYSIS OF VARIANCE OF RESPONSE OF *Lonicera tartarica* TO A SERIES OF INDOLYL ACIDS

Source of variance	Degrees of freedom	Mean square					
		Number of cuttings rooted (transformed data)[s]	Length of roots per cutting rooted	Mean root length, mm.	Number of roots per cutting rooted	Green weight of leaves	Fresh weight of roots
Replicates	3	479.0	17499	120.3	15.74	18.18	1.064
Dosages	2	6.8	51050	236.7	34.07	2.24	0.027
Error (a)	6	671.2	45636	121.7	27.40	18.40	2.557
Chemicals	5	173.7	168639***	149.7**	411.47***	14.42*	3.108**
Chemicals × dosages	10	136.0	22814	65.8	23.76	7.64	1.418
Error (b)	45	190.9	18182	42.3	25.93	4.25	0.787

S *Data subjected to inverse sine transformation (1).*
* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

Table II shows the effect of chemicals, as estimated from the differences between treated and control means. Indolylbutyric acid increased the length and number of roots per rooted cutting and the fresh root weight, but decreased the mean root length and the green weight of leaves. Indolylacetic acid increased the length and number of roots per rooted cutting. Indolyl-

propionic acid decreased the mean root length and the green weight of leaves. Neither 5-methyl-indolylpropionic nor indolylvaleric acids had any significant effect on the responses studied.

TABLE II

RESPONSE OF *Lonicera tartarica* TO A SERIES OF INDOLYL ACIDS

Treatment	Number of cuttings rooted (transformed data)[S]	Length of roots per cutting rooted, mm.	Mean root length, mm.	Number of roots per rooted cutting	Green weight of leaves, gm.	Fresh weight of roots, gm.
Control	55.7	241	30	8	8.6	1.23
Indolylacetic	55.2	377*	30	12*	7.4	1.58
Indolylpropionic	49.3	260	23*	11	6.4*	0.84
5-Methyl-indolylpropionic	51.4	234	25	10	7.4	1.23
Indolylbutyric	56.9	544*	24*	24*	5.6*	2.21*
Indolylvaleric	59.8	321	31	11	8.0	1.95
Necessary difference for 5% of significance		111	5.3	4	1.7	0.73

S *Data subjected to inverse sine transformation (1).*
* *Exceeds 5% level of significance.*

Indolylbutyric acid was the most consistently effective of the chemicals employed; and the general effect of chemicals was to increase the number while decreasing the individual length of roots, and to increase total root weight while reducing the weight of tops.

The results would appear to indicate that members of the indolyl series with an even number of carbon atoms in the side chain have greater physiological activity than those with an odd number, a conclusion in essential agreement with the variation of activity in the naphthyl series. However, while there was little difference in the activity of naphthylbutyric and -acetic acids, in the indolyl series the butyric acid was markedly more active than the acetic homologue.

Acknowledgment

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VEGETATIVE PROPAGATION OF CONIFERS

III. EFFECT OF MONTH OF COLLECTION ON THE ROOTING OF DORMANT NORWAY SPRUCE CUTTINGS[1]

By N. H. Grace[2]

Abstract

In a comparison of the rooting responses of cuttings of Norway spruce from upper branches, in which one lot was stored under snow from November until early April and the other collected in late March, there were statistically significant differences. After 12 weeks 38.9% of the November, and 20.7% of the March, cuttings were rooted or calloused. At the end of the propagation period 45.2% of the November, and 66.6% of the March, cuttings were dead. Indolylacetic acid dust treatment did not have a significant effect on the number of cuttings rooted or calloused; it did, however, increase the number that were dead at the end of the experiment.

Among the factors of possible importance in the vegetative propagation of conifers are the effects of period of collection and storage on the rooting of dormant cuttings. Deuber and Farrar have shown recently that there is a marked change in the rooting of cuttings of *Picea excelsa* Link. over the period of October to January (2). The present communication describes a preliminary experiment in which the effect of time of collection on the rooting of Norway spruce cuttings was studied.

Experimental

Branches of Norway spruce were collected in mid-November 1938 and buried under snow until early April (3, 4). These were compared for rooting ability with a collection of branches made in March 1939.* Both collections were from the upper part of trees from a plantation approximately 18 years of age, on the Petawawa Forest Experiment Station, Chalk River, Ontario. Cuttings ranged from two to four inches in length and had a heel of old wood, and were divided into groups of 13 cuttings, each representative of the various lengths. Prior to planting, four replicates of 13 cuttings of each collection were treated with talc only, with 100, and with 1000 p.p.m. (parts per million) of indolylacetic acid in talc. There was also an untreated control.

The cuttings were planted April 5, 1939, in brown sand in a propagation frame equipped with bottom heat cables. The propagation frame was provided with a factory cotton screen, which reduced light intensity and assisted in maintaining the humidity. The random order of planting resulted

in one level of precision for all the comparisons. Sand temperature was maintained at 72° F., while the room temperature ranged between 65 and 70 F°. for the first few weeks of the propagation period. However, during the last eight weeks the room temperature frequently went up to from 80 to 95 °F. during the day.

The cuttings were removed 12 weeks after planting and the number rooted, calloused, and dead, and the number and length of roots were determined. Poor rooting of the March collection rendered it impossible to make a statistical analysis of the data for the number and length of roots per rooted cutting. This led to combination of the number of cuttings calloused with those rooted for statistical analysis, based on inverse sine transformations (1).

Results

Data for the number of cuttings rooted or calloused and for the number dead were subjected to analyses of variance, and the results are presented in Table I. The number of cuttings rooted or calloused was affected by the

TABLE I

ANALYSES OF VARIANCE OF RESPONSE OF NORWAY SPRUCE CUTTINGS COLLECTED IN NOVEMBER AND MARCH AND TREATED WITH DUSTS CONTAINING INDOLYLACETIC ACID

| | | Mean square | |
Source of variance	Degrees of freedom	Number of cuttings calloused or rooted	Number of cuttings dead
Replicates	3	136.24	49.82
Indolylacetic acid dosage	3	260.59	238.32*
Month of collection	1	1309.44***	1352.00***
Interaction			
Indolylacetic acid treatment × month of collection	3	102.37	296.37*
Error	21	86.93	66.62

* Exceeds mean square error, 5% level of significance.
*** Exceeds mean square error, 0.1% level of significance.

month of collection, but neither indolylacetic acid treatment nor the interaction between indolylacetic acid treatment and month of collection had a significant effect. The number of cuttings dead showed that the month of collection was significant to the 0.1% level, and indolylacetic acid treatment and the interaction between treatment and month of collection also were significant, but only to the 5% level.

In Table II are given data for the effects of the month of collection on the number of cuttings rooted or calloused and the number dead. The November

TABLE II

THE EFFECT OF MONTH OF COLLECTION ON THE NUMBER OF DORMANT NORWAY SPRUCE
CUTTINGS ROOTED OR CALLOUSED

Month of collection of cuttings	Cuttings			
	Rooted or calloused		Dead	
	Transformed data	Per cent	Transformed data	Per cent
November	38.2	38.9	42.1	45.2
March	24.4	20.7	55.1	66.6
Necessary difference, 5% level	6.9		6.1	

collection gave significantly more rooted or calloused cuttings, and fewer
dead cuttings than the March collection.   There were 19.7% of the November
cuttings rooted, while only 2.9% of the March collection rooted.

The effects of indolylacetic acid treatment and the month of collection on
the number of cuttings that died are shown in Table III.   Both 100 and
1000 p.p.m. treatments increased mortality of November cuttings over the
untreated control; the untreated and talc groups did not differ.   The response
of March cuttings to treatment was somewhat different in that 1000 p.p.m.
indolylacetic acid caused significantly greater mortality than was shown by
the untreated or 100 p.p.m. groups.   The interaction effect was due to this
difference in response of cuttings of the two collections to 100 p.p.m. indolyl-
acetic acid treatment; in March, this treatment, while not differing significantly
from the untreated control, suggested a decrease in mortality.   The treat-
ment means indicated that 1000 p.p.m. indolylacetic acid in talc increased
mortality over the untreated group; values for the other two treatments were
intermediate but did not differ significantly from the latter.

TABLE III

EFFECTS OF MONTH OF COLLECTION AND INDOLYLACETIC DUST TREATMENT ON THE
NUMBER OF NORWAY SPRUCE CUTTINGS DEAD

Month of collection of cuttings	Untreated	Indolylacetic acid in talc, p.p.m.			Necessary difference, 5% level
		0	100	1000	
Transformed data					
November	34.7	36.6	49.6	47.3	12.0
March	50.7	60.1	45.1	64.5	
Treatment means	42.7	48.4	47.4	55.9	8.5

The results are in general agreement with those of Deuber and Farrar, and suggest that there is a marked change in rooting ability during the dormancy period (2). It would appear that branches stored under snow, following collection in November, do not undergo physiological changes to the same extent as if left on the tree throughout the winter. It may not be assumed that such storage completely stops all physiological change, since November cuttings, the collection used in this experiment, responded favourably to 1000 p.p.m. indolylacetic acid in talc when treated in December (4). However, close comparisons cannot be drawn between the former and present experiment, since in the one the cuttings were plain and in the other they had a heel of old wood. Further, propagation conditions varied markedly owing to summer temperatures.

It would appear that the rooting of dormant Norway spruce cuttings depends to a greater extent on the period of collection than on treatment with root growth stimulating chemicals.

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THE EFFECT OF LEAF RUST ON THE YIELD AND QUALITY OF THATCHER AND RENOWN WHEAT IN 1938[1]

By B. Peturson[2] and Margaret Newton[3]

Abstract

A study was made at Winnipeg in 1938 to determine the effect of leaf rust on the yield and quality of Thatcher and Renown wheat. In one experiment, Thatcher and Renown were sown late in 1/400-acre plots; in another, Thatcher only was used and was sown early in rod-row plots. Half the plots of each variety were kept as free from leaf rust as possible by frequent applications of sulphur dust, but the remaining half became heavily infected. In the 1/400-acre plots, leaf rust reduced the yield of Thatcher and Renown by 51.17 and 29.61%, respectively; in the rod-row plots of Thatcher, it reduced the yield by 37.02%. The decrease in yield was due more to reduction in kernel weight than to reduction in number of kernels per head. All the non-dusted plots ripened approximately three days earlier than the dusted, and the grain from them graded one grade lower than that from the corresponding dusted plots. In both varieties, the protein content was diminished while the carotene content was increased.

Stem rust (*Puccinia graminis Tritici* Erikss. & Henn.) and leaf rust (*Puccinia triticina* Erikss.) have caused a great deal of damage to the wheat crop in the Prairie Provinces of Canada. Generally speaking, when stem rust has been severe on wheat, leaf rust has been somewhat abundant on it also. For this reason, in studies (2) already made in the Prairie Provinces on the effect of stem rust and leaf rust on the yield and quality of wheat, an exact allocation of the damage caused by either of these rusts could not be made. Undoubtedly a great proportion of the injury has been attributable to stem rust, as Marquis, the common wheat formerly chiefly grown, was only moderately susceptible, and Mindum, the only durum variety widely grown, has been rather resistant to leaf rust.

That leaf rust of wheat is capable of doing extensive damage has already been shown. Caldwell *et al.* (1), Hayes *et al.* (3), Johnston (4), Johnston and Miller (5), Mains (6), Melchers (7), and Waldron (9) in the United States, and Phipps (8) in Australia have shown that leaf rust very materially reduced the yield of wheat.

With the introduction into the Prairie Provinces of new varieties of wheat resistant to stem rust, some of which are quite susceptible to leaf rust, an opportunity was afforded for ascertaining to what extent the new wheats might be injured by leaf rust. Experiments were planned, therefore, to determine the effect of leaf rust on the yield and quality of two of the new varieties resistant to stem rust, namely, Thatcher and Renown.

Materials and Methods

Two different experiments were carried out. In the first, the varieties Thatcher and Renown were grown on summer-fallowed land in 1/400-acre plots. There were 12 plots of each variety. Half the plots of each variety were kept as free from rust as possible by dusting them three times a week, from June 23 to August 4, with sulphur (Kolodust) at the rate of 30 lb. per acre per dusting. The remaining plots were not dusted. The plots were not artificially inoculated with leaf rust but were sown late (May 23) in order to permit the rust to develop as fully as possible on them. Both varieties were sown at the rate of 1½ bu. per acre.

In the second experiment the variety Thatcher only was used. It was sown early (May 11) in rod-row plots. Each plot consisted of three rod-rows spaced one foot apart and sown on summer-fallowed land at the rate of 500 seeds per row. There were in all 25 pairs of plots. During the third week in June, one plot of each pair was artificially inoculated with leaf rust. The non-inoculated plots of each pair were dusted at the same rate and time as those in the first experiment. The centre rows only of each plot were harvested.

In both experiments, leaf rust percentages were estimated while the leaves were still green, stem rust percentages, just before the plants ripened. The estimate of rust percentages was based on the scale of stem-rust percentages adopted by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture. For each individual plot in both experiments, the yield, number of kernels per head, bushel weight, and 1,000-kernel weight were taken, and, through the courtesy of the Western Grain Inspection Division, Winnipeg, commercial grades were obtained on the bulked dusted and non-dusted samples of each variety. The bulked samples of grain from the dusted and the non-dusted plots of both experiments were each tested for baking quality.

Experimental Results

Severity of Infection in Plots

A severe outbreak of leaf rust of wheat occurred in Manitoba in 1938. All the non-dusted plots of Thatcher, both in the artificially and in the naturally infected plots, became heavily rusted; those of Renown were less heavily rusted. In the 1/400-acre plots of Thatcher, the infection averaged 68 and 32% in the non-dusted and dusted plots, respectively; in the Renown plots, 33% in the non-dusted plots and 13% in the dusted plots. In the rod-row plots of Thatcher, leaf-rust infection averaged 77% in the non-dusted plots, and 32% in the dusted plots. Only very slight traces of stem rust developed in the Thatcher and Renown plots. That is to say, a few pustules occurred at the nodes of a small percentage of the plants. These pustules were not sufficiently numerous to have any appreciable effect on the yield of the two varieties, either in the dusted or non-dusted plots.

Effect of Leaf Rust on Yield, Weight per Measured Bushel, and Grade of Thatcher and Renown

The grade, yield, and weight per measured bushel of the dusted and non-dusted 1/400-acre plots of Thatcher and Renown, and the rod-row plots of Thatcher are given in Table I. From these results it is evident that leaf rust of wheat reduced the yield of the 1/400-acre plots of Thatcher and Renown (sown late), and of the rod-row plots of Thatcher (sown early). In the 1/400-acre plots, the non-dusted Renown yielded 29.61% less than the dusted, and the non-dusted Thatcher, 51.17% less than the dusted. In the rod-row plots, the non-dusted Thatcher yielded 37.02% less than the dusted. Thatcher was, therefore, more seriously affected than Renown and when sown late was damaged appreciably more than when sown early.

TABLE I

THE EFFECT OF LEAF RUST ON THE YIELD, BUSHEL WEIGHT, AND GRADE OF THATCHER AND RENOWN GROWN IN FIELD PLOTS AT WINNIPEG IN 1938

Type of plot	Variety	Treatment	Time of sowing	Leaf rust, %	Average yield per acre, bu.	Decrease in yield per acre due to leaf rust, %	Average weight per bushel, lb.	Grade (Northern)
1/400-acre	Thatcher	Dusted	Late-sown**	31	23.86	51.17	62.10	No. 2
		Non-dusted	Late-sown**	68	11.65		56.80	No. 3
	Renown	Dusted	Late-sown**	13	24.75	29.61	64.10	No. 1
		Non-dusted	Late-sown**	33	17.42		62.20	No. 2
Rod-row	Thatcher	Dusted	Early-sown*	32	31.56	37.02	64.60	No. 2
		Non-dusted	Early-sown*	77	19.86		61.00	No. 3

*Sown May 11.   **Sown May 23.

Leaf rust also influenced the length of the ripening period. Although both the dusted and non-dusted plants of early-sown Thatcher came into head on the same day, the non-dusted plants ripened three days earlier than the dusted plants.

The weight per measured bushel and grade of both these varieties were also adversely affected by leaf rust. The grain from the non-dusted plots of early-sown Thatcher, late-sown Thatcher, and Renown plots weighed 3.6, 5.3, and 1.9 lb. per bu. less, respectively, than the grain from the corresponding dusted plots. In each case the grain of the non-dusted plots graded one grade lower than that of the corresponding dusted plots.

The Effect of Leaf Rust on Kernel Weight and Number of Kernels per Head

Reduction in yield by leaf rust of wheat may be brought about in different ways. Mains (6) found that, in Illinois, reduction was due to two main

causes: *(a)* the tip and basal spikelets of the head, as well as other spikelets in the remaining portion of the head, often failed to set seed with the result that the number of kernels per head was reduced; and *(b)* the individual kernels did not attain their normal size and plumpness. In Kansas, Johnston and Miller (5) found that reduction in grain yield was due, primarily, to the production of fewer kernels by rusted plants and, secondarily, to reduced kernel weight. Similarly, Caldwell *et al.* (1) in Illinois, and Johnston (4) in Kansas, found that reduction in yield was due mainly to a reduction in the number of kernels per head. Waldron (9), on the other hand, found that, under field conditions in North Dakota, the reduction in yield from leaf rust was due mainly to a reduction in the weight of the kernels.

These discrepancies in results may be explained, in part at least, by the fact that the degree of kernel reduction may depend on the stage of development of the plants at the time of the onset of the rust. Mains (6) pointed out that when infection occurred relatively early, reduction in yield was due chiefly to a reduction in the number of kernels, but when infection occurred late, to a reduction in kernel weight and, to a much less extent, to a reduction in number of kernels. The explanation of this change appears to be that if severe rust infection occurs sometime before fertilization takes place, the number of seeds formed may be materially reduced, whereas, if the infection takes place after fertilization occurs, the number of seeds produced may not be greatly reduced.

An attempt was made, therefore, to determine at Winnipeg if the early-sown Thatcher, infected by leaf rust at a late stage, would show a smaller reduction in number of kernels than the late-sown Thatcher, infected at an early stage; and if the reduction in yield in the early-sown Thatcher would be due chiefly to a reduction in kernel weight, while that of the late-sown Thatcher and Renown would be due chiefly to a reduction in number of kernels. The loss in kernel weight was determined by obtaining the weights of 1,000-kernel lots, taken at random from the threshed grain samples of the individual dusted and non-dusted plots, both of the early and late-sown plots. The number of kernels produced per head was determined by ascertaining the average number of kernels per head of plants selected at random from the outside rows of the dusted and non-dusted plots. For this purpose, 20 heads were taken from each plot.

The results presented in Table II show that in the early-sown Thatcher (infected at heading time) there was a reduction in number of kernels of 7.00%, while in the late-sown Thatcher (infected two weeks before heading) there was a reduction of 17.41%. However, as will be seen in Table II, the reduction in yield in the early-sown Thatcher, the late-sown Thatcher, and Renown, sown at the same time as late-sown Thatcher, were due more to a reduction in kernel weight than to reduction in number of kernels. For example, the reduction in kernel weight of the grain in the non-dusted plots of the early-sown Thatcher, the late-sown Thatcher, and Renown amounted to 26.47, 27.08, and 16.16%, respectively, whereas the reduction in number of kernels

per head in the same plots amounted to 7.00% in the early-sown Thatcher, 17.41% in the late-sown Thatcher, and 5.74% in Renown. That is to say, in the early-sown Thatcher, 77.8% of the loss in yield in the non-dusted plots was due to reduction in kernel weight and the remaining 22.2% to reduction in number of kernels per head. In the late-sown Thatcher and in Renown, reduction in kernel weight amounted to 56.2 and 66.6%, respectively, and reduction in number of kernels to 43.8% in Thatcher and 33.4% in Renown.

TABLE II

THE EFFECT OF LEAF RUST ON THE WEIGHT PER 1,000 KERNELS AND NUMBER OF KERNELS PER
HEAD OF THATCHER AND RENOWN GROWN IN FIELD PLOTS AT WINNIPEG IN 1938

Type of plot	Variety	Treatment	Time of sowing	Average weight per 1,000 kernels, gm.	Decrease in weight per 1,000 kernels due to leaf rust, %	Average number of kernels per head	Decrease in number of kernels per head due to leaf rust, %
1/400 acre	Thatcher	Dusted	Late-sown**	22.63	—	22.40	17.41
		Non-dusted	Late-sown**	16.50	27.08	18.50	
	Renown	Dusted	Late-sown**	30.01	—	17.75	5.74
		Non-dusted	Late-sown**	26.36	16.16	16.73	
Rod-row	Thatcher	Dusted	Early-sown*	27.81	—	22.36	7.00
		Non-dusted	Early-sown*	20.45	26.47	20.79	

*Sown May 11.   **Sown May 23.*

In these trials, the epidemic of leaf rust in the late-sown plots did not reach a maximum until the plants were almost in head, and, in the early-sown plots, until after the plants were in head. Had the attack of leaf rust become severe at a very early stage in the life of the plants, the ratio of loss in number of kernels to loss in weight of kernels would probably have been different. In view of the fact that, in the Prairie Provinces, leaf rust seldom becomes well established until the wheat plants are almost in head, it seems probable that, as a rule, the reduction in yield by leaf rust is due more to reduced size than to reduced number of kernels.

All the data pertaining to yield, bushel weight, and 1,000-kernel weight, but not those pertaining to baking and milling tests, were subjected to statistical analysis, and the differences were found to be significant.

Effect of Leaf Rust on the Milling and Baking Qualities of Thatcher and Renown

Grain samples from the dusted and non-dusted plots of Thatcher and Renown were submitted for quality tests to the Grain Research Laboratory of the Board of Grain Commissioners, Winnipeg, and to the Cereal Division, Experimental Farms Service, Ottawa. As the results of these two tests

TABLE III

MILLING AND BAKING TESTS CONDUCTED BY THE CEREAL DIVISION, EXPERIMENTAL FARMS BRANCH, OTTAWA
(MALT-PHOSPHATE-BROMATE METHOD)

Type of plot	Sample	Treatment	Wheat protein, %	Flour yield, %	Flour colour (carotene), p.p.m.	Absorption, %	Dough character	Loaf volume, cc.	Loaf form	Crust[1] colour	Crumb[1] texture	Crumb[1] colour
1/400 acre	Late-sown Thatcher	Dusted	14.5	74.0	2.48	59	Strong	847	5	5	8.6	7.3
		Non-dusted	14.0	71.7	3.00	60	Strong	877	5	5	8.0	5.6
	Late-sown Renown	Dusted	15.5	73.4	2.15	60	Strong	921	5	5	8.3	7.6
		Non-dusted	15.3	73.0	2.53	59	Strong	914	5	5	8.3	7.3
Rod-row	Early-sown Thatcher	Dusted	15.6	72.0	2.25	61	Strong	818	5	5	8.4	7.4
		Non-dusted	14.6	72.6	2.97	61	Strong	854	5	5	8.0	5.6

[1] *Maximum scores: Loaf form 5; Crust colour 5; Crumb texture 10; Crumb colour 10.*

were in agreement, it seems necessary to include only one set of data.   That furnished by the Cereal Division is presented in Table III.   The results show that the grain from the dusted plots was higher in protein content, but lower in carotene content, than that from the non-dusted plots.   The crumb colour of the bread baked from wheat from the dusted plots, particularly the Thatcher plots, was superior to the crumb colour of that baked from the wheat from the non-dusted plots.   With Renown wheat, the loaf volume was greater in the samples from the dusted plots than from the non-dusted plots.   The reverse, however, was true of Thatcher.   In all the other milling and baking characteristics, the grain from the dusted and non-dusted plots gave very similar results.

Discussion

A true measure of the damage caused by leaf rust of wheat was not obtained in these experiments as leaf rust was not completely controlled in the dusted plots.   If leaf rust had been suppressed completely in these plots, the differences in yield and quality of the grain from the dusted and non-dusted plots would undoubtedly have been greater.   These experiments, however, clearly show that, under severe leaf-rust infection such as prevailed throughout most of Manitoba in 1938, leaf rust materially reduced the grade, yield, and quality of Thatcher and Renown, and that Thatcher, the more susceptible of these two varieties, suffered greater damage than Renown.   In all these tests the differences in grade, yield, and quality of grain from corresponding dusted and non-dusted plots can be attributed to the difference in amount of leaf rust on the plants and not to any direct beneficial effect of sulphur to the plants.   Experiments conducted by Greaney (2) at Winnipeg have shown that, in the absence of rust and other leaf and stem diseases, the dusting of wheat varieties with sulphur during the growing period has no appreciable effect on yield.

In the present experiment, the rusted plants ripened three days earlier than those kept free from rust by sulphur dust.   This shortening of the ripening period can probably be attributed to the effect of the fungus on the wheat plants, and also to a lack of moisture in the soil, for, at Winnipeg, during the growing season of 1938, the precipitation was abnormally low.   Weiss (11) studied the water requirement of Marquis wheat when infected with stem rust and leaf rust, and found that the rusted plants had a higher water requirement than the non-rusted ones, although the differences were significant only in the case of stem-rust infection.   Weaver (10) compared the rate of transpiration in rusted and non-rusted wheat, rye, barley, and oats, and found that the transpiration rate was consistently higher in the rusted plants. Johnston and Miller (5) found that, in the greenhouse, when heavy leaf-rust infection occurred early in the growth of the plants, the water requirement of a susceptible wheat variety was more than doubled.   It would seem, therefore, that, in the field experiments at Winnipeg, the rusted plants did not have a large enough water supply to meet their increased needs, and, consequently, matured early.   In the greenhouse experiments by Johnston

and Miller (5), the rusted plants were abundantly supplied with water and ripened later than those free from rust. Some observations made by the writers at different times seem to indicate that when wheat plants are infected with leaf rust, but well supplied with water, the maturity appears to be delayed. It is just possible that, had the precipitation at Winnipeg in 1938 been excessive, leaf rust might have had the opposite effect, namely, to delay the ripening of the rusted plants.

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EFFECTS OF TEMPERATURE AND SUNLIGHT ON THE RATE OF ELONGATION OF STEMS OF MAIZE AND GLADIOLUS[1]

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Abstract

Measurements of the height of the main stems of maize and gladiolus plants were made at four-hour intervals starting at 4 a.m. The increases in elongation were analyzed statistically, together with mean temperature, hours of sunlight, and mean relative humidity.

Partial correlation coefficients showed that there was a highly significant positive association between the rate of stem elongation (growth) and temperature, regardless of the time of day, the variations in temperature accounting for from 40 to 70% of the variability in growth rates. There was likewise a significant negative correlation between growth and sunlight, but sunlight was apparently effective only during the midday periods (8 a.m. to 4 p.m.). The depressing effect of sunlight on the growth of gladiolus was approximately four times as great as on maize. This depressing effect on maize was entirely removed by shading the plants with light white cotton.

Variations in relative humidity were only slightly associated with growth rates. No significant effect was observed for any of the periods. It seems possible that these factors might be more important under conditions of deficiency in soil moisture.

The approximate minimum temperature at which growth took place was 40° F.

Introduction

Many years ago it was pointed out by Reed (22) that although much study had been given to the effects of external factors on total growth, little had been given to the effects of the same factors on rate of growth. Since that time there has been a considerable amount of work carried out in an effort to determine the effects of external factors on rate of growth, but no general agreement as to the relative importance of the different factors has been reached. The factors most generally considered are temperature, sunlight, relative humidity, and rainfall. The last mentioned is related to the internal condition of the plant and also plays a part in determining relative humidity. If soil moisture is not limiting, the latter effect is probably the more important.

Probably more attention has been given to the effects of temperature than to the effects of any other factor, although many investigators have considered sunlight as an important factor in determining rate of growth. With respect to the relative magnitude of the effects of these two factors two extremes of opinion are illustrated by the statement of Maximov (15) and the work of Porterfield (19, 20). Maximov states (p. 135): "The retarding influence of light on growth is so great that it creates a definite daily period-

icity. During the night, plants grow more rapidly than during the day, in spite of the lower nocturnal temperatures". The statement apparently applies to all plants, the only exception noted having to do with very sharp drops in nocturnal temperatures which result in stunted plants. Porterfield (20), on the other hand, found a consistently higher rate of growth in the bamboo *(Phyllostachys nigra)* during the day than during the night.

In the following sections no attempt is made to give a complete review of the literature, but representative work is cited. It must be noted at the outset that a large proportion of the data pertinent to the present study has been obtained by measuring elongation of a particular organ rather than by measuring increase in total growth as shown by dry weights. Bakhuyzen (1) considers that since elongation is a unidimensional measure, it does not give as accurate an estimation of growth as does dry weight. There is some indication that the two measures do not give the same type of results, at least with particular material. Bakhuyzen, however, considers that measurements of elongation of wheat leaves and internodes yield typical growth curves, although the first part of the curve is usually missed in actual measurements.

The results of all studies show increased growth with increased temperatures (9, 13, 15, 17). For example, Leitch (9) found that the elongation of pea roots immediately after germination was directly dependent on temperature, the curve being a straight line between 12 and 29° C. If this straight line is extrapolated it cuts the line of zero growth at 3° C., while experimental results showed this point to be at −2° C. The minimum temperature at which growth takes place varies with the plant. Values for maize and wheat are given as 5 to 10° C. and 0 to 5° C. respectively (15); and for maize as 49° F. (17). The results of Leitch (9) show that such values must be determined experimentally, since extrapolation from results at higher temperatures does not always give a reliable figure.

The effect of light has not been as definitely determined. Sunlight certainly retards the growth of many plants (6, 8, 10, 14, 18, 21), and it is apparently the shorter wave-lengths that are effective (14, 25). Studies with various species of bamboo have shown that growth of some is retarded during hours of bright sunlight (10, 18), while that of others is not (19, 20).

Many of the studies carried out have been concerned with the relative rate of growth by day and night. There has been no uniformity in the division of the day into these two periods, however, and a fair comparison of results is rendered difficult. In general it must be concluded that the greater growth was obtained at night (10, 12, 14, 16, 18, 21, 24), but definite exceptions to this result have been noted (19, 20). It must further be concluded, however, that the data secured are often inadequate to separate the effects of the various environmental conditions.

Some investigators (2, 10, 11, 16, 24) regard the greater growth by night as a result of moisture relations rather than of the inhibiting effect of sunlight. This conclusion has been reached in various ways, but in few cases has it been substantiated by experiments under controlled conditions or by results sub-

jected to statistical analysis. Loomis (11), however, did get a definite effect when the moisture supply was controlled.

The work of Gregory (6) on the growth of barley is more like the present study than any others with which we are familiar. The measurements of growth used are different, but the results obtained were subjected to a statistical analysis. Partial correlations showed that growth was positively and significantly correlated with day temperatures, but negatively and usually significantly correlated with bright sunlight. Most of the correlation coefficients involving evaporation data were not significant, but Gregory states that the primary data could not be considered satisfactory.

The present study was undertaken with the hope that more definite information might be obtained as to the rate of growth of plants by day and night, and as to the relative importance of the various external factors affecting growth. All data were collected with the intention of using them in a statistical analysis, the results of which should show definitely the relative importance of the various factors under our local conditions.

Material and Methods

The studies reported in this paper were carried out at Edmonton, Alberta, during the summers of 1937 and 1938. The more important measurements were made with maize and gladiolus plants, although in the first year wheat was included in the study. These plants were selected because the stems all possess a well-defined terminal point, which makes accurate measurement relatively easy, they all grow rapidly enough to give growth figures that are high in comparison with the error of reading, and the elongation should be a fair measure of growth, since growth in this case is largely unidimensional. Maximov (15) states that in annual cereals elongation does not begin until all internodes and inflorescences have been laid down, and Bakhuyzen (1) found that the elongation of the two upper internodes takes place at the same time. The "growth" of the wheat plants that we measured was, therefore, the elongation of the cells of the upper internodes of the stems. This is probably true of maize as well, but in gladiolus a somewhat different situation exists. The first elongation results from the lengthening of the flower spike below the first floret. After the spike is well out of the sheath, elongation between florets, beginning at the lowest one, takes place. It seemed possible that this latter elongation might upset the essentially linear relations between elongation and external factors, but such proved not to be the case. As will be seen later, there is no evidence that elongation of the stem as a whole did not proceed very regularly.

Conditions at Edmonton are particularly favourable for separating the effects of temperature and sunlight. Night temperatures are usually considerably lower than those by day, and the total range of temperatures during an experiment is usually high. In this latitude (53½° N) the maximum possible sunlight during the time most of these measurements were made

is almost 15 hr. a day. The division of the day into three eight-hour periods starting at 4 a.m. gives two daylight periods and one dark period.

The material used in this study and the series designations were as follows:—

Wheat I, 1937. Variety Red Bobs, seed obtained from the pure-seed plots of this Department. Measurements made on 29 plants at 4 a.m., noon and 8 p.m. from July 13 to July 22.

Wheat II, 1937. Variety Red Bobs. Measurements made on 30 plants at the same times from July 19 to July 27.

Maize, 1937. An early sweet hybrid (I_M–34–1), developed in this Department. Measurements made on 24 plants at the same times from July 17 to July 25.

Gladiolus I, 1937. Mixed varieties. Measurements made on 20 plants at the same times from July 22 to August 1.

Gladiolus II, 1937. Mixed varieties. Measurements made on 25 plants at 4 a.m., 8 a.m., noon, 4 p.m. and 8 p.m. from August 7 to August 15.

Unshaded maize, 1938. Same variety as in 1937. Measurements made on 30 plants every four hours starting at 4 a.m. from July 19 to July 28.

Shaded maize, 1938. Ten plants in the same plot and for the same periods as those of the preceding series. During the day these plants were shaded on two sides and from above with white cotton cloth. Air movement was but slightly reduced, and air temperatures in the daytime were only about 1° F. higher than in the open. The shades were removed at night.

Gladiolus I, 1938. Variety Bit O' Heaven. Measurements made on 25 plants at 4 a.m., 8 a.m., noon, 4 p.m. and 8 p.m. from July 24 to July 30.

Gladiolus II, 1938. Variety Picardy. Measurements made on 20 plants at 4 a.m., 8 a.m., noon, 4 p.m. and 8 p.m. from August 2 to August 10.

The wheat and all gladiolus series were grown in a garden which was unshaded by trees or buildings. The maize was grown in a similar garden in 1937, but in an open field at the University of Alberta in 1938.

The periods of the day are designated as follows: 4 a.m. to 8 a.m., morning; 8 a.m. to noon, early midday; noon to 4 p.m., late midday; 4 p.m. to 8 p.m., evening; and 8 p.m. to 4 a.m., night.

Before measurements were begun, small stakes with squared tops were driven into the ground close to the stem to be measured. All measurements were made with a metre stick, graduated in millimetres, placed squarely on the top of the stake. The tip of the stem (head, tassel or flower spike) was held firmly, but without strain, against the stick and the reading made to the nearest millimetre. Measurements were begun as soon as the tip appeared.

In 1937 no records of temperature, sunlight, etc., were kept for the exact locations at which the plants were grown. The data used are those recorded at the Edmonton station of the Dominion Meteorological Service. In 1938, continuous records were kept in the field in which maize was grown. These

records were compared with those of the Dominion station, and agreed so well that the latter have been used throughout this paper in order to make the results of the two years uniform.

In arriving at the mean temperature and mean relative humidity, hourly readings from continuous records were averaged. Sunlight records were obtained with a Campbell-Stokes sunshine recorder. The data so obtained are inadequate for evaluating the intensity factor of sunlight, but were the best obtainable.

The accuracy of the growth measurements was determined by carrying out an analysis of variance for the data obtained for 10 consecutive readings on 25 plants. The error so determined includes both the personal error of observation and the interaction of individual plant growth with time. The standard error for the mean of 25 plants (Gladiolus I, 1938) was 0.056 cm. The corresponding value for the personal error only (determined on the results of four replicate readings made at one time) was 0.022 cm.

Results

Individual data, or even means for individual periods, are much too numerous to include in this paper. The presentation and discussion of the actual data are confined to a minimum, and in the graphs only data from single series are given. These results illustrate general effects, the specific importance of each one being much more clearly determined by the results of the statistical analysis. In this paper, elongation of the stem is termed "growth".

ACTUAL DATA

A summary of the mean growth and temperature data for eight-hour periods in all series is presented in Table I. Growth was in general proportional to temperature, the agreement being closest for maize, and least for gladiolus.

TABLE I

MEAN GROWTH AND TEMPERATURE FOR EIGHT-HOUR PERIODS

Series	No. of days	8 p.m. to 4 a.m.		4 a.m. to noon		Noon to 8 p.m.	
		Growth, cm.	Temperature, °F.	Growth, cm.	Temperature, °F.	Growth, cm.	Temperature, °F.
Wheat I, 1937	7	1.08	59.6	1.17	60.8	1.33	67.9
Wheat II, 1937	6	1.07	56.3	1.37	61.0	1.72	69.3
Maize, 1937	7	0.94	58.7	1.60	63.2	2.55	71.6
Gladiolus I, 1937	10	0.99	54.5	1.23	57.3	1.46	66.5
Gladiolus II, 1937	9	0.98	54.0	1.31	57.9	1.18	64.8
Unshaded maize, 1938	9	1.16	58.6	1.47	63.2	2.50	75.8
Shaded maize, 1938	9	1.17	58.6	1.66	63.2	2.62	75.8
Gladiolus I, 1938	6	1.62	56.2	1.66	62.2	2.19	74.4
Gladiolus II, 1938	8	1.18	50.0	1.10	54.0	1.66	60.8

This relation is illustrated in Fig. 1, which shows the results for individual eight-hour periods for the unshaded maize, 1938. Included in this graph are sunlight and relative humidity values. There were only traces of rainfall during the ten days.

There are exceptions to this relation between growth and temperature, however, and the exceptions are greatly accentuated when results for four-hour periods are considered. The results for the four daylight periods are presented in Table II, and in Fig. 2, together with data for sunlight, humidity, and rainfall. These results show a reversal of the general growth-temperature
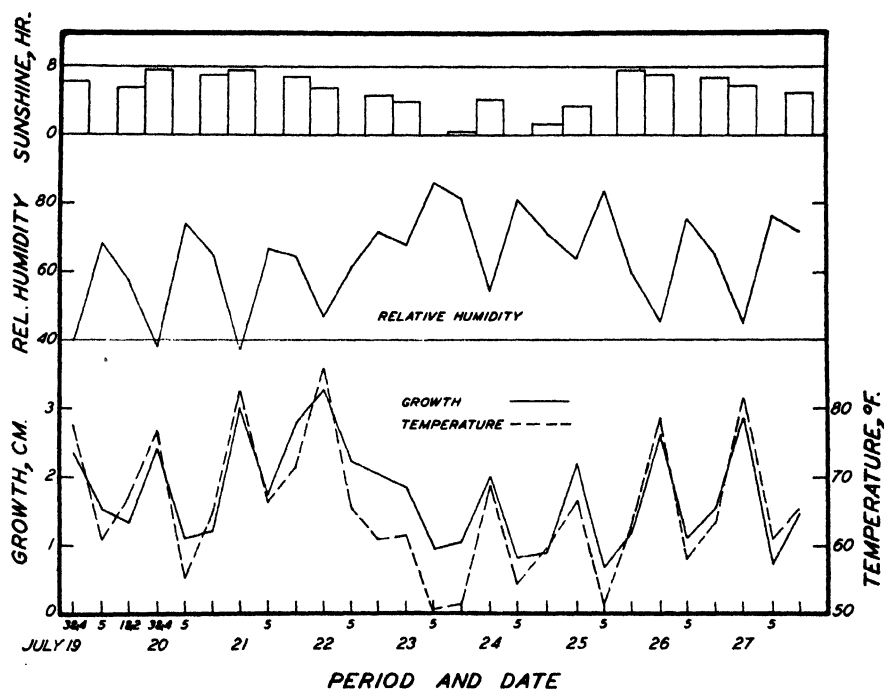
Fig. 1. *Unshaded maize, 1938. Growth during 8-hr. periods in relation to external factors. Periods are numbered as follows: 1, morning (4 a.m. to 8 a.m.); 2, early midday (8 a.m. to noon); 3, late midday (noon to 4 p.m.); 4, evening (4 p.m. to 8 p.m.); 5, night (8 p.m. to 4 a.m.).*

relation when there was bright sunlight during the middle of the day. When there was no sunshine, however, growth was roughly parallel to temperature. While these effects were much more evident with gladiolus than with maize, the conclusions are applicable to both plants.

Sunlight depressed growth much less during the morning and evening periods than duri ng the middle of the day. The results in Table II and Fig. 2 do not show th t sunlight had no effect during these periods, but it is fairly evident from Fig. 3 that only during the midday periods was the depressing effect appreciab e. Fig. 3 is for the second gladiolus series, 1938, and the points for each period are clearly defined. The figures beside the points indicate the hours of sunlight per four-hour period. Since no midnight

readings were taken, the points for the night period are plotted as half the total growth against the mean temperature for the eight hours. This method merely reduces the total number of entries, but makes no assumptions regarding the distribution of growth during the two halves of the eight-hour period. The growth for every midday period during which sunlight was recorded was depressed below the general growth-temperature line as determined for the other periods. The growth figures for the midday periods on days when the sun did not shine are in good agreement with those for other periods. Regardless of the duration of direct sunlight, growth during the morning and evening periods was apparently not affected in the way it was during the midday periods. This conclusion will later be shown to be justified by the results of the statistical analysis.

There is an apparent concentration of growth readings for the morning and night periods above (relatively) those for the other periods, regardless of sunlight. Whether this is significant or not cannot be determined from the present data, but it seems possible that the results for the midday and evening periods are affected by some factor not accounted for in this work. This conclusion is supported by the position of the two regression lines shown

TABLE II

MEAN GROWTH, TEMPERATURE AND SUNLIGHT DURING THE 4-HOUR DAYLIGHT PERIODS

Series	No. of days	Morning (4 a.m. to 8 a.m.)			Early midday (8 a.m. to 12 noon)		
		Growth, cm.	Temperature, °F.	Sunlight, hr.	Growth, cm.	Temperature, °F.	Sunlight, hr.
Gladiolus II, 1937	9	0.73	52.8	1.5	0.48	63.1	2.3
Unshaded maize, 1938	9	0.52	55.6	2.0	0.95	70.8	3.0
Shaded maize, 1938	9	0.49	55.6	2.0	1.17	70.8	3.0
Gladiolus I, 1938	6	0.93	55.2	1.9	0.73	69.2	2.8
Gladiolus II, 1938	8	0.65	48.9	1.3	0.45	59.0	2.1

Series	No. of days	Late midday (12 noon to 4 p.m.)			Evening (4 p.m. to 8 p.m.)		
		Growth, cm.	Temperature, °F.	Sunlight, hr.	Growth, cm.	Temperature, °F.	Sunlight, hr.
Gladiolus II, 1937	9	0.37	66.9	2.5	0.81	62.7	1.2
Unshaded maize, 1938	9	1.02	78.2	3.5	1.48	73.5	2.1
Shaded maize, 1938	9	1.39	78.2	3.5	1.24	73.5	2.1
Gladiolus I, 1938	6	0.74	77.2	2.9	1.45	71.6	1.7
Gladiolus II, 1938	8	0.68	63.5	1.4	0.97	58.2	0.9
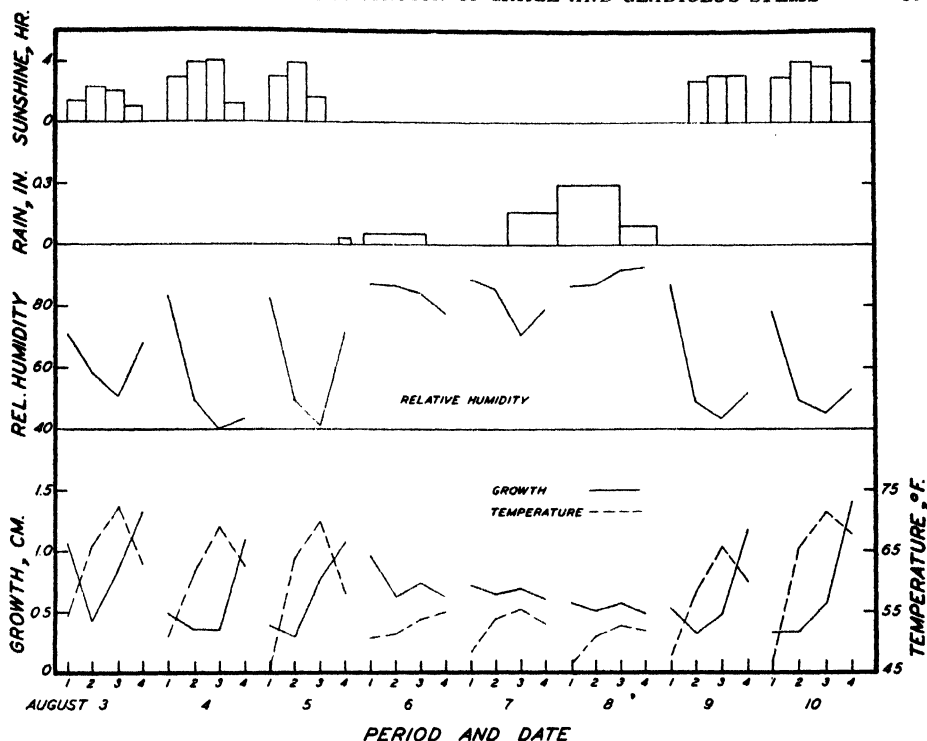
FIG. 2. Gladiolus II, 1938. Growth during 4-hr. daylight periods in relation to external factors. Periods are numbered as in Fig. 1.

(anticipated from the data and discussion in the next section). Allowing for the effect of sunlight did not account for the entire difference in the relative levels of growth during the midday periods as compared with the others. This may be the result of inadequate sunlight data or increased error in temperature data at the higher levels. Certainly it would be expected that the temperatures of the meristems would be further from air temperatures on a bright warm day than on a cool one. If the temperatures recorded during sunny hours were higher than the temperatures of the meristems, the effect would be as shown in Fig. 3.

A number of other factors may have been operative in determining the magnitude of the midday readings, but since it is impossible to determine which were important, extended discussion does not seem warranted.

The failure of the two regression lines to coincide when the effect of sunlight was removed raises a question with regard to the linearity of the effects of temperature and sunlight on growth rate. The data obtained with Shaded Maize, 1938, and with Gladiolus II, 1938 (the series shown in Fig. 3) were tested for non-linearity. The deviations from the linear regression line were not significant for either the growth-temperature or growth-sunlight relation. Thus the failure to get better agreement between the two regression lines is probably due to deficiencies in the data or to some factor not here considered.
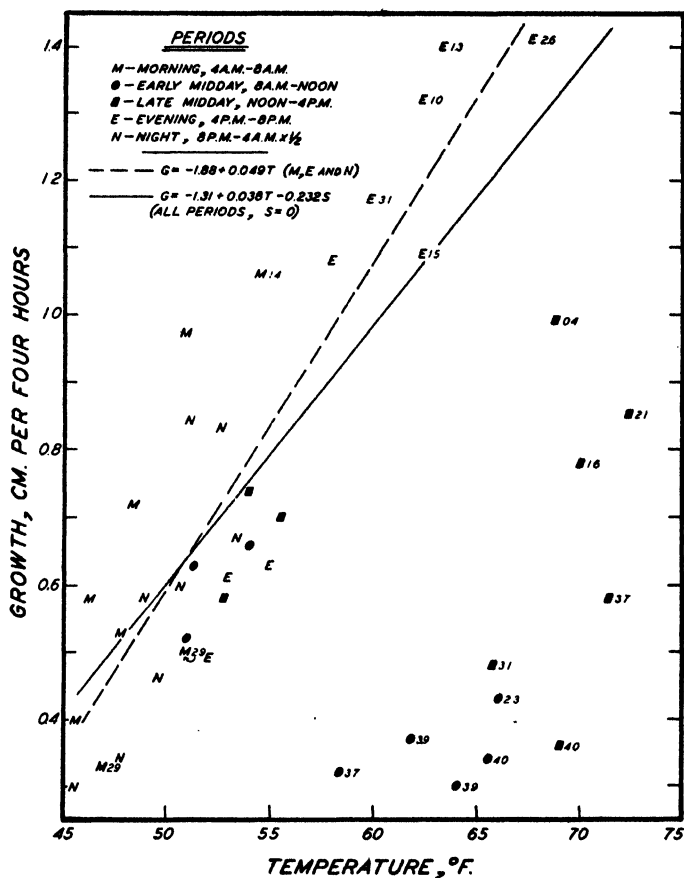
FIG. 3. *Gladiolus II, 1938. The effects of temperature and sunlight on growth per 4 hr. The figures beside the symbols denote hours of sunlight per 4-hr. period.*

Whether 8 a.m. and 4 p.m. mark the extremes of time at which sunlight exerts a depressing effect on growth is doubtful. Measurements were made at 6 p.m. and 10 p.m. in addition to the regular times on July 26 (Gladiolus I, 1938). The results on the basis of hourly growth, together with temperature and sunlight values, are given in Table III. These data can be considered to

TABLE III

MEAN HOURLY GROWTH OF GLADIOLUS FROM NOON, JULY 26 TO 4 A.M., JULY 27

Time	Growth, cm.	Temperature, °F.	Sunlight, hr.
12 noon to 4 p.m.	0.12	80.5	0.95
4 p.m. to 6 p.m.	0.24	81.0	0.95
6 p.m. to 8 p.m.	0.58	72.5	0.70
8 p.m. to 10 p.m.	0.30	65.0	—
10 p.m. to 4 a.m.	0.21	56.2	—

give only an indication, but they suggest that the depressing effect of sunlight may be felt later than 4 p.m. Presumably the same effect might have been apparent during the morning period. Further discussion is hardly warranted here, since only one day's results are available.

STATISTICAL ANALYSIS OF THE DATA

The methods of statistical analysis used in the following sections are fully outlined by Goulden (5) and Snedecor (23). The dependent variable is always growth (g, the increase in length), and the independent variables studied are temperature (t), sunlight (s), and relative humidity (h). Rainfall is omitted from the calculations, since there was none during the time several of the series were studied, and because preliminary examination showed that this factor was not of primary importance. The number of periods represented for each series and each time is given in Table IV.

TABLE IV

THE NUMBER OF INDIVIDUAL PERIODS REPRESENTED FOR EACH SERIES AND EACH TIME

Series	All periods	Daily totals	8 a.m. to 4 p.m.	4 p.m. to 8 p.m.	8 p.m. to 4 a.m.
Wheat I, 1937	–	7	–	–	8
Wheat II, 1937	–	6	–	–	7
Maize, 1937	–	7	–	–	8
Gladiolus I, 1937	–	10	–	–	11
Gladiolus II, 1937	45	8	18	9	8
Maize, 1938	54	9	20	9	9
Shaded maize, 1938	54	9	20	9	9
Gladiolus I, 1938	31	6	12	6	6
Gladiolus II, 1938	42	8	17	9	8

Temperature Effects

The simple and partial correlation coefficients obtained with the temperature data for all series are presented in Table V. All the simple coefficients are positive and most of them significant. There is, however, a consistency in those that are not significant, since all but two of them are for gladiolus, and most of them are for data that include results of the midday periods. Holding the effects of sunlight and humidity constant in general increased the correlation between growth and temperature, and brought the results for gladiolus into agreement with those for wheat and maize.

Gladiolus I, 1938, gave lower partial correlation coefficients than did any other series. Measurements of .this series were carried out for only six days, and variations in external conditions during this time were much less than with most of the other series. More extended measurements would probably have made the results for this series significant.

The effect of temperature on the two series of maize plants grown in 1938 was similar. The only difference in the two series was that the shaded plants

TABLE V

RELATION BETWEEN TEMPERATURE AND GROWTH OF WHEAT, MAIZE, AND GLADIOLUS AS MEASURED BY SIMPLE AND PARTIAL CORRELATION COEFFICIENTS

Series	All periods	Daily totals	8 a.m. to 4 p.m.	4 p.m. to 8 p.m.	8 p.m. to 4 a.m.
Simple correlation coefficients, r_{gt}					
Wheat I, 1937	–	0.821*	–	–	0.824**
Wheat II, 1937	–	0.865*	–	–	0.723
Maize, 1937	–	0.987**	–	–	0.921**
Gladiolus I, 1937	–	0.939**	–	–	0.929**
Gladiolus II, 1937	0.256	0.780*	0.069	0.811**	0.613
Maize, 1938	0.751**	0.891**	0.548*	0.859**	0.782*
Shaded maize, 1938	0.897**	0.863**	0.717**	0.810**	0.638
Gladiolus I, 1938	0.152	0.588	0.000	0.346	0.829*
Gladiolus II, 1938	0.289	0.670	0.067	0.941**	0.838**
Partial correlation coefficients, $r_{gt \cdot sh}$					
Wheat I, 1937	–	0.722	–	–	0.831*a
Wheat II, 1937	–	0.882*	–	–	0.726a
Maize, 1937	–	0.995**	–	–	0.921**a
Gladiolus I, 1937	–	0.940**	–	–	0.935**a
Gladiolus II, 1937	0.823**b	0.791*	0.736**b	0.747*b	–
Maize, 1938	0.602**	0.909**	0.639**	0.908**	−0.321a
Shaded maize, 1938	0.620**	0.725*	0.586*	0.883**	−0.071a
Gladiolus I, 1938	0.340	0.433	0.937**	0.694	0.666a
Gladiolus II, 1938	0.647**	0.872*	0.808**	0.826*	0.841*a

a *Coefficient $r_{gt \cdot h}$, as there was no sunlight during these periods.*
b *Humidity data not available.*
Significant beyond the 5% point.
**Significant beyond the 1% point.*

were protected from the direct sun by light white cotton. The results for the night period (8 p.m. to 4 a.m.) with the two series are not in agreement with the others in Table V. The negative partial correlation coefficients cannot be accepted as a true indication of the effect of temperature on growth, and must be attributed to some peculiarity of the data involved.

The general conclusion reached as a result of the correlation analyses is that there is a significant positive effect of temperature on the growth of these three plants, and that in many instances this effect accounts for a large proportion of the variability found in the growth rates.

Sunlight Effects

The simple and partial correlation coefficients involving the sunlight data for all series are presented in Table VI. It should be emphasized again that when all periods are considered together, the sunlight data for midday periods only were included in the correlation analysis.

Only a few of the simple correlation coefficients are significant, but they exhibit a fairly consistent behaviour. For most of the gladiolus series the

TABLE VI

RELATION BETWEEN HOURS OF SUNLIGHT AND GROWTH OF WHEAT, MAIZE, AND GLADIOLUS AS MEASURED BY SIMPLE AND PARTIAL CORRELATION COEFFICIENTS

Series	All periods[a]	Daily totals	8 a.m. to 4 p.m.	4 p. m. to 8 p.m.
Simple correlation coefficients, r_{os}				
Wheat I, 1937	–	0.580	–	–
Wheat II, 1937	–	0.675	–	–
Maize, 1937	–	0.205	–	–
Gladiolus I, 1937	–	0.352	–	–
Gladiolus II, 1937	−0.398**	0.228	−0.331	0.474
Maize, 1938	0.157	0.525	0.154	0.286
Shaded maize, 1938	0.615**	0.681*	0.567	0.394
Gladiolus I, 1938	−0.542**	−0.414	−0.951**	−0.330
Gladiolus II, 1938·	−0.415**	−0.178	−0.667**	0.695*
Partial correlation coefficients, $r_{os·th}$				
Wheat I, 1937	–	−0.150	–	–
Wheat II, 1937	–	0.721	–	–
Maize, 1937	–	−0.809	–	–
Gladiolus I, 1937	–	−0.390	–	–
Gladiolus II, 1937	−0.842**[b]	−0.321[b]	−0.769**[b]	0.000[b]
Maize, 1938	−0.600**	−0.624	−0.030	−0.163
Shaded maize, 1938	0.163	0.001	0.345	0.414
Gladiolus I, 1938	−0.776**	−0.160	−0.995**	−0.898*
Gladiolus II, 1938	−0.841**	−0.692	−0.944**	−0.063

[a] *Sunlight during the midday periods only.*
[b] *Humidity data not available.*
Significant beyond the 5% point.
**Significant beyond the 1% point.*

coefficients are negative, while for maize they are all positive, though not significant. The significant effect of sunlight on the growth of Shaded Maize, 1938, disappears when the effects of temperature and humidity are removed. Thus sunlight affected growth of these shaded plants only through its effect on the other environmental conditions.

The partial correlation coefficients are consistently negative except for the daily totals for one wheat series. This result cannot be explained, but it is not significant. The results show that the depressing effect of sunlight on growth was more pronounced with gladiolus than with maize. They likewise show that only for those analyses in which results for midday periods were included was this depressing effect significant. There is one exception with Gladiolus I, 1938, but even though the negative correlation coefficient was significant, the regression coefficient was much smaller than that for the midday periods.

The effect of sunlight on daily growth was negative but not significant. Since the number of days involved was small, the coefficients must be very

high to be significant, and more extended series would probably show a significant depressing effect of sunlight on total daily growth. This conclusion is supported by the results for shaded maize plants, which on bright sunny days made an average growth of 6.6 cm. per day as compared with 5.8 cm. for the unshaded plants. This difference is significant, and cannot be attributed to temperature differences, since temperature under the shade was not more than 1° F. higher than the temperature in general.

These results show that bright sunlight exerts a depressing effect on growth during the midday periods, and that this effect is more pronounced with gladiolus than with maize. They do not permit a final conclusion as to the effects during other periods, but these, if any, are small compared with the effects during the middle of the day. There is a strong indication that total daily growth is also depressed by bright sunlight.

Humidity Effects

The simple and partial correlation coefficients involving humidity data are presented in Table VII. Very little need be said regarding these results, since only one of the partial coefficients is significant.

TABLE VII

RELATION BETWEEN RELATIVE HUMIDITY AND GROWTH OF WHEAT, MAIZE, AND GLADIOLUS AS MEASURED BY SIMPLE AND PARTIAL CORRELATION COEFFICIENTS

Series	All periods	Daily means	8 a.m. to 4 p.m.	4 p.m. to 8 p.m.	8 p.m. to 4 a.m.
Simple correlation coefficients, r_{gh}					
Wheat I, 1937	–	−0.484	–	–	−0.226
Wheat II, 1937	–	−0.889*	–	–	0.032
Maize, 1937	–	−0.621	–	–	−0.378
Gladiolus I, 1937	–	0.170	–	–	0.066
Gladiolus II, 1937	–	–	–	–	–
Maize, 1938	−0.656**	−0.674*	−0.225	−0.475	−0.902**
Shaded maize, 1938	−0.829**	−0.689*	−0.545	−0.437	−0.710*
Gladiolus I, 1938	0.003	0.105	0.754	−0.375	−0.662
Gladiolus II, 1938	−0.161	−0.130	0.165	−0.794*	−0.045
Partial correlation coefficients, $r_{gh\cdot t s}$					
Wheat I, 1937	–	0.023	–	–	0.289
Wheat II, 1937	–	−0.018	–	–	−0.099
Maize, 1937	–	0.182	–	–	0.463
Gladiolus I, 1937	–	0.107	–	–	0.280
Gladiolus II, 1937	–	–	–	–	–
Maize, 1938	0.011	0.502	0.375	0.502	−0.756*
Shaded maize, 1938	0.099	0.452	0.256	0.672	−0.409
Gladiolus I, 1938	−0.221	0.121	0.384	−0.620	0.040
Gladiolus II, 1938	−0.080	−0.027	−0.083	−0.170	0.139

*Significant beyond the 5% point.
**Significant beyond the 1% point.

The significant simple correlation coefficients are all negative and are significant only because humidity was highly and negatively correlated with temperature. When the effect of temperature was removed the relation between growth and humidity becomes negligible in most series.

The only significant partial coefficient is also negative. This result cannot be accepted as biologically significant, and since it is for the same series as that which yielded the negative relation between growth and temperature, the peculiarity of the data is emphasized.

In the present study there is no evidence that relative humidity has any effect in determining the rate of growth. However, since this conclusion applies to fluctuations in relative humidity independent of those associated with temperature differences, it is possible that real effects of atmospheric humidity may have been confounded with temperature effects.

Multiple Correlation Coefficients

The multiple correlation coefficients summarizing the combined effects of temperature, sunlight, and humidity are given in Table VIII. Most of these are highly significant and show that when results for all periods are considered together (sunlight for midday periods only), from 65 to 80% of the variability in growth can be accounted for in terms of temperature, sunlight, and humidity. For individual periods and daily means an even greater proportion of the variability in growth results is accounted for.

TABLE VIII

COMBINED EFFECT OF TEMPERATURE, SUNLIGHT,[a] AND RELATIVE HUMIDITY ON THE GROWTH OF WHEAT, MAIZE, AND GLADIOLUS AS MEASURED BY MULTIPLE CORRELATION COEFFICIENTS

Series	All periods $R_{g \cdot tsh}$	Daily means $R_{g \cdot tsh}$	8 a.m. to 4 p.m. $R_{g \cdot tsh}$	4 p.m. to 8 p.m. $R_{g \cdot tsh}$	8 p.m. to 4 a.m. $R_{g \cdot th}$
Wheat I, 1937	–	0.825	–	–	0.840*.
Wheat II, 1937	–	0.990**	–	–	0.727
Maize, 1937	–	0.996**	–	–	0.937**
Gladiolus I, 1937	–	0.997**	–	–	0.935**
Gladiolus II, 1937	0.854**[b]	0.803[b]	0.769**[b]	0.784[b]	–
Maize, 1938	0.855**	0.933**	0.661*	0.933*	0.913**
Shaded maize, 1938	0.900**	0.864	0.761**	0.907*	0.711
Gladiolus I, 1938	0.803**	0.872	0.995**	0.916	0.830
Gladiolus II, 1938	0.861**	0.914*	0.969**	0.941**	0.841*

[a] *Midday periods only.*
[b] $R_{g \cdot ts}$, *humidity values not available.*
Significant beyond the 5% point.
**Significant beyond the 1% point.*

Regression of Growth on Temperature and Sunlight

The partial regression coefficients for growth on temperature and sunlight for all series and periods are presented in Table IX. The results for all periods

together are the most reliable since the number of entries for individual periods is small, and one result which was not in agreement with the general trend could, and in some cases did, alter the regression coefficients unduly.

TABLE IX

PARTIAL REGRESSION COEFFICIENTS OF GROWTH OF WHEAT, MAIZE, AND GLADIOLUS ON TEMPERATURE AND SUNLIGHT

Series	All periods cm./4 hr./°F.	Daily totals cm./day/°F.	8 a.m. to 4 p.m. cm./4 hr./°F.	4 p.m. to 8 p.m. cm./4 hr./°F.	8 p.m. to 4 a.m.[a] cm./8 hr./°F.
Growth on temperature, $b_{gt \cdot sh}$					
Wheat I, 1937	–	0.230	–	–	0.059
Wheat II, 1937	–	0.151	–	–	0.074
Maize, 1937	–	0.261	–	–	0.079
Gladiolus I, 1937	–	0.230	–	–	0.078
Gladiolus II, 1937	0.035[b]	0.158[b]	0.030[b]	0.039[b]	0.083[b]
Maize, 1938	0.044	0.312	0.023	0.044	0.069[c]
Shaded maize, 1938	0.039	0.196	0.030	0.042	0.059[c]
Gladiolus I, 1938	0.027	0.114	0.019	0.011	0.111
Gladiolus II, 1938	0.038	0.300	0.025	0.064	0.123
Growth on sunlight, $b_{gs \cdot th}$ (cm. per hr. sunshine)					
Wheat I, 1937	–	−0.040	–	–	–
Wheat II, 1937	–	0.081	–	–	–
Maize, 1937	–	−0.055	–	–	–
Gladiolus I, 1937	–	−0.041	–	–	–
Gladiolus II, 1937	−0.206[b]	−0.032[b]	−0.090[b]	0.000[b]	–
Maize, 1938	−0.049	−0.169	−0.051	−0.005	–
Shaded maize, 1938	0.007	−0.003	0.069	0.146	–
Gladiolus I, 1938	−0.208	−0.035	−0.105	−0.030	–
Gladiolus II, 1938	−0.232	−0.125	−0.153	−0.003	–

[a] $b_{gt \cdot s}$ since there was no sunlight at night.

[b] $b_{gt \cdot s}$ and $b_{gs \cdot t}$ since humidity data not available.

[c] simple regression coefficients.

The temperature effect was fairly uniform, an increase of 1° F. increasing the growth per four hours by about 0.04 cm. The results for daily totals are for 24 hr., and when reduced to a four-hour basis agree reasonably well with the results for all periods. Similarly the results for the night periods (8 p.m. to 4 a.m.) are for eight hours, and agree well on the four-hour basis except for the last two series, which are higher than the other coefficients obtained.

The correlation results were subjected to a covariance analysis, splitting the correlation into within and between periods. With only one series was there any significant difference between the regression coefficients for the different periods, and this difference disappeared when one irregular value

was omitted. It is concluded, therefore, that the correlation involving temperature is homogeneous for all periods.

There is a pronounced variability in the regression coefficients involving sunlight, however, the results for the evening period yielding insignificant coefficients while those for all periods and for midday periods are relatively high, particularly for the gladiolus. Bright sunlight for four hours during the middle of the day depressed the growth of gladiolus approximately 0.8 cm. below that expected at any specified temperature. This means that the temperature had to be about 24° F. higher during periods of bright sunlight than during the other periods to give the same increase in growth. The corresponding figure for maize is about 4.5° F.

The fact that the regression coefficients for the midday periods alone are lower than those for all periods requires an explanation. The magnitude of these coefficients depends not only on the degree of correlation, but also on the magnitude of the standard deviations of growth and sunlight readings. During the midday periods alone, the variability in growth rates was low, because warm days were sunny and cool days were not. For example, with Gladiolus II, 1938, the standard errors for growth during midday and all periods were respectively 0.203 and 0.959, while those for temperature during the same periods were 7.52 and 7.96, and those for sunlight were 1.41 and 1.60. Thus, even if the correlations were higher during midday periods the regression coefficients would be lower than those for all periods.
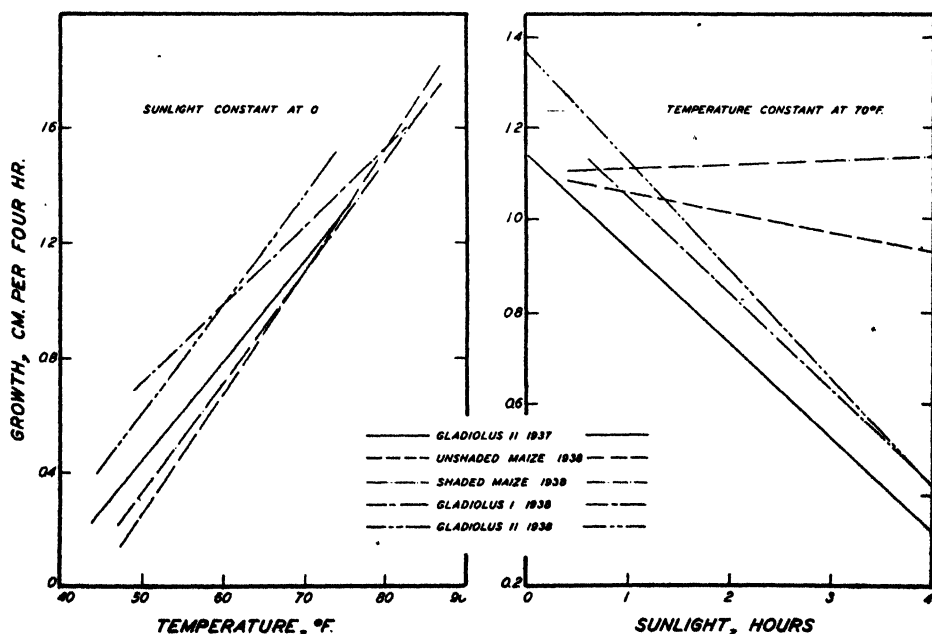
· FIG. 4. *Regression of growth on temperature and sunlight. Partial regressions holding the second independent variable constant. Results for all individual periods. Lines cover only the temperature and sunlight ranges actually obtained experimentally.*

The general independent effects of temperature and sunlight (sunlight effects for midday periods only) are shown in Fig. 4. These two graphs show the growth expected as expressed in terms of temperature or sunlight under constant conditions of the other independent variable. Under any other specific conditions than those shown, for example sunlight constant at four hours or temperature constant at 60° F., the slope of each line, and the relation among all lines would be unaltered, but the numerical value of the growth increases would be changed.

TEMPERATURE COEFFICIENTS

Many biological reactions do not obey the van't Hoff law with respect to increased rates with increasing temperature. The work of Leitch (9) has already been referred to, and while he did not calculate Q_{10} values, it can readily be seen that his results would yield steadily falling values as temperature increased. Only at about 13 to 23° C. was this value equal to 2.0. The present study offered an opportunity to calculate Q_{10} values at various levels. The results of such calculations for the temperature increase from 15 to 25° C., based on partial regression coefficients, are presented in Table X. Sunlight and humidity values are taken equal to the averages obtained for each series. Where extrapolation was necessary to obtain a value for 25° C. the result is marked with an asterisk. There is no real difference between the values obtained from series in which no extrapolation was necessary and those in which it was. The mean Q_{10} for the former is 2.04 and for the latter 1.98.

TABLE X

TEMPERATURE COEFFICIENTS FOR THE TEMPERATURE INCREASE 15 TO 25° C. BASED ON PARTIAL REGRESSION COEFFICIENTS

Series	All individual periods	Daily totals	4 p.m. to 8 p.m.	8 p.m. to 4 a.m.
Wheat I, 1937	–	2.31	–	1.98
Wheat II, 1937	–	1.88*	–	2.00*
Maize, 1937	–	2.01	–	2.39
Gladiolus I, 1937	–	2.08*	–	2.04*
Gladiolus II, 1937	1.84	–	2.03	2.07*
Unshaded maize, 1938	2.22	2.14	1.90	1.97*
Shaded maize, 1938	2.10	1.83	2.12	1.82*
Gladiolus I, 1938	–	–	–	2.11*
Gladiolus II, 1938	1.72	1.73*	2.15*	1.97*

Involves extrapolation beyond temperatures actually obtained experimentally.

The effect of temperature on the temperature coefficients for Unshaded Maize, 1938, at different periods of the day is illustrated in Fig. 5. The range

covered by these curves involves no extrapolation of regression lines. In each instance (except 8 p.m. to 4 a.m.) the partial regression coefficient has been used, the other independent variables being held constant at the mean value obtained experimentally. The variations at lower temperatures are due to the differences in the point at which no growth is calculated to take place. At higher temperatures the agreement is excellent.
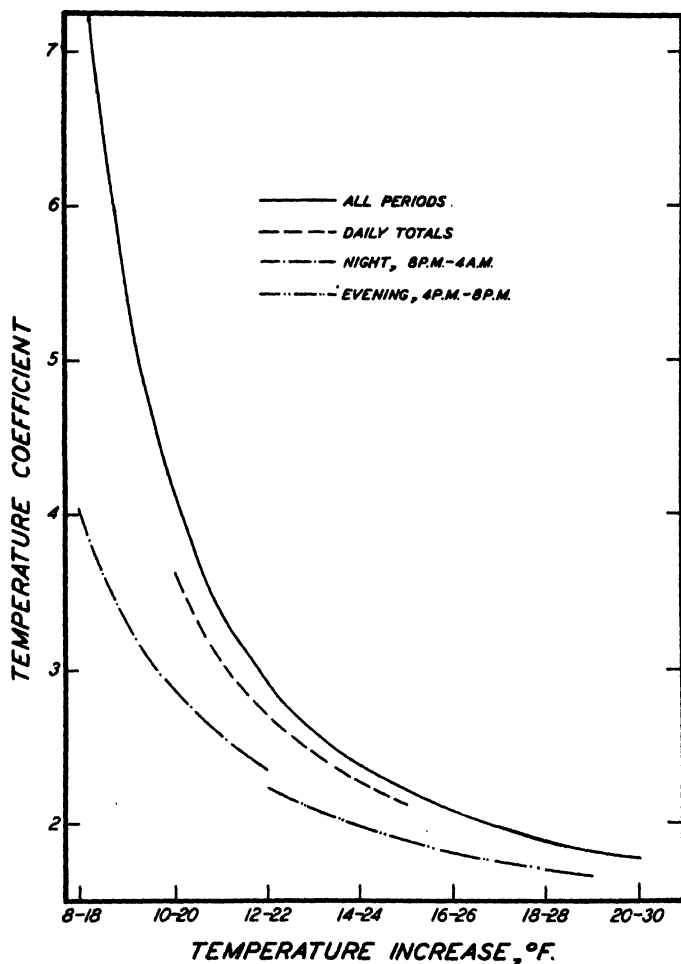
FIG. 5.   *Unshaded maize, 1938.   Temperature coefficients in relation to temperature level.*

It has frequently been remarked, of course, that since growth is not a single reaction it cannot be expected to obey any simple rule. It is not implied in this discussion that the Q_{10} values express the effect of temperature on a single reaction, but rather on the whole complex mechanism that results in stem elongation. That the values obtained should be as consistent as they are is remarkable when the enormous variations in other factors are considered.

Approximate Minimum Temperature at Which Growth Took Place

By extrapolation of the regression lines for growth in terms of temperature, a measure of the minimum temperature at which growth took place can be obtained. The values for all data with which significant correlation co-efficients were obtained are given in Table XI. Again the other variables have been held constant at the means obtained experimentally.

TABLE XI

ESTIMATED MINIMUM TEMPERATURES (° F.) AT WHICH GROWTH TOOK PLACE. BASED ON PARTIAL REGRESSION COEFFICIENTS

Series	All periods	Daily totals	Evening period	Night period
Wheat I, 1937	–	45.4	–	40.6
Wheat II, 1937	–	37.6	–	–
Maize, 1937	–	41.2	–	40.5
Gladiolus I, 1937	–	42.3	–	41.7
Gladiolus II, 1937	37.6	35.7	41.6	–
Unshaded maize, 1938	44.3	43.2	39.1	40.5
Shaded maize, 1938	41.6	37.1	42.9	–
Gladiolus I, 1938	22.8	–	–	42.8
Gladiolus II, 1938	34.2	39.3	43.3	40.4

It has already been noted that such extrapolation is invalid in determining the exact level at which growth takes place. The relative uniformity of the results in Table XI, however, indicates that there is a distinct similarity in the behaviour of the three plants studied. The results for all periods should be the most reliable, since they are obtained from the most extensive data. They likewise involve the least extrapolation except for the night periods with which the same amount would be required. These values indicate that the gladiolus will grow at a lower temperature than will maize. The result for Gladiolus II, 1938, is low because the regression coefficient is low. This flattens the regression line with a consequent greater extension being necessary before it cuts the growth axis. Although none of these values can be considered exact, they are in fairly good agreement with those reported by others (15, 17). The results obtained with maize are a few degrees lower than others reported, but there may be a difference with different varieties.

Discussion

The results presented in this paper show conclusively that the rate of elongation of stems of maize, gladiolus, and wheat is affected by atmospheric temperature and sunlight. With most of the series, the variations in temperature and sunlight during individual periods account for from 60 to 90%

of the variability in growth. The effect of humidity is less well defined, but it appears to be relatively unimportant under the conditions of our experiments. All of these plants had an adequate supply of moisture during the time measurements were taken, and it seems possible that the humidity might be more important under conditions of moisture deficiency.

It seemed possible that a closer association between growth and external factors might be obtained if an allowance for delayed effect were made. Growth for individual periods was correlated with temperatures for the preceding as well as for the actual periods, that is allowing a four-hour lag in growth, but in general the association was not improved by this procedure. The calculations were repeated allowing a two-hour lag with the same result. It is concluded, therefore, that there is a fairly rapid response to changes in external temperature. The same general result was obtained with sunlight.

One of the primary objects of this study was to determine whether the plants grew faster by day or by night. This question can only be answered by specifying the external conditions. Undoubtedly if the day were bright and sunny, growth of gladiolus from 6 a.m. to 6 p.m. would be less than from 6 p.m. to 6 a.m. unless the temperature during the latter period was relatively low. On the other hand, on a dull day growth from 6 a.m. to 6 p.m. would be greater if the mean temperature were higher. For many of the series, the amount of elongation which would take place under specific conditions could be accurately calculated from a knowledge of temperature and sunlight values. Thus with Gladiolus II, 1938, elongation during the night (dark period) was as great at 60° F. as it was during the midday periods (with continuous bright sunshine) at 84° F.

If, however, we consider the hourly rate of growth during daylight (4 a.m. to 8 p.m.) and during darkness, the former was always the higher. The daylight hours include the evening period, during which actual growth was usually most rapid, since temperature was usually high and sunlight apparently ineffective. There was a sufficient decrease in temperature at night to more than offset the fact that there was no retarding effect of light. In other words, the total retarding effect of light from 4 a.m. to 8 p.m. was less than the retarding effect of the lower temperatures at night.

The effect of sunlight on gladiolus was much more pronounced than that on maize. Nevertheless, the shading experiment with maize showed that growth was greater for the shaded plants, both during the bright-light periods and for the daily totals. The differences could not be due to higher temperature under the shades, since this was never more than 1° higher than in the open.

The results of this study are concerned with the effects of external factors. Although air temperatures should be more or less closely related to the temperature of the meristems, the latter should give a closer association with growth. The relation of sunlight to growth is quite different, since the internal expression of this external factor is not accounted for. It is the light of short wave-length that is active in depressing growth (14, 25), and the difference

in behaviour of different plants might be the result of a difference in the ease of penetration of the waves concerned to the meristems. This is particularly likely if the retarding effect of the light is due to a reduction of sensitivity of cells to auxin action (25).

The results obtained by Gregory (6) are of interest to us because his measures of growth more nearly fulfil the conditions specified by Bakhuyzen (1) as essential for a determination of real growth rates than do ours. The results of his studies with relative growth rates of leaves agree with the results of the present study, although his correlation coefficients were in general lower. It seems likely, therefore, that the conclusions reached as a result of this study may be applicable to growth in general, as well as to stem elongation.

The results here presented offer a possible explanation of some of the experimental results obtained by other workers. Lock (10) considered the day period as between 7.30 a.m. and 5.30 p.m., and found that the growth rate of bamboo was much greater by night than by day. There is every reason to believe that this division of the day would result in an exaggerated difference between night and day growth, since the early and late hours of sunlight, during which growth may be rapid, are excluded from the day period. Of course, the exaggeration would be much less in the tropics, where Lock's work was done, than in northern latitudes. Lock likewise correlated growth with rainfall, but it seems possible that the relation between growth and sunlight might have been just as important. There would be no direct sunlight when it was raining, and if temperature remained constant, growth should be greater when the sun was not shining.

Prescott (21) found two maxima in the rate of growth of maize. These are of interest because they occurred just after sunrise and just before sunset, and our results indicate that similar maxima would have been obtained with hourly measurements. MacDougal (12) found retardation in the growth rate of wheat and maize occurred after 11 a.m. In the present study it began before this, although the exact point cannot be determined from the data obtained. The results obtained by MacDougal may well have been due to sunlight, although he does not mention this factor.

Hanna (7) obtained positive correlations between growth of maize and sunflowers and temperature, and between growth and hours of sunshine. Measurements were made at two- or three-day intervals and only simple correlation coefficients were calculated. Some of Hanna's conclusions are outside the field of this paper, but it seems probable that partial correlations would have shown that the positive relation between growth and sunlight was due to the relation between sunlight and temperature.

Extremes of altitude affect growth of plants, owing partly to temperature differences but also to light effects (15). It seems probable that if not only the duration and intensity, but also the composition of the sunlight were known, a more complete explanation of results obtained could be made. It seems probable that during sunlight hours much less ultraviolet light

would be incident on plants when the atmosphere was moist than when it was relatively dry. If conditions could be controlled so that relative humidity could be varied independently of temperature and sunlight, it might be shown to be more important than it now appears to be.

Controlled environmental conditions are essential for the most accurate determination of the effects of individual factors on the rate of growth. Under such conditions, the effect of light of varying composition could be studied, as could any one factor under constant conditions of others. Unless facilities are available for such control, however, it is believed that the statistical analysis used in this paper offers the most precise method available for separating the effects of the various factors involved in determining growth rates.

Acknowledgments

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A DESCRIPTIVE LIST OF NATURAL AND ARTIFICIAL INTERSPECIFIC HYBRIDS IN NORTH AMERICAN FOREST-TREE GENERA[1]

By L. P. V. JOHNSON[2]

Abstract

Over 400 hybrids involving 28 North American genera of forest trees are described in tabular form with the object, primarily, of providing useful information for the forest-tree breeder. The genera involved are: *Abies*, *Acer*, *Aesculus*, *Alnus*, *Arbutus*, *Betula*, *Carya*, *Castanea*, *Catalpa*, *Cornus*, *Crataegus*, *Cupressus*, *Gleditsia*, *Ilex*, *Juglans*, *Larix*, *Magnolia*, *Picea*, *Pinus*, *Platanus*, *Populus*, *Quercus*, *Robinia*, *Salix*, *Taxus*, *Tilia*, *Tsuga*, and *Ulmus*.

Introduction

For many years botanists have been describing natural hybrids, or to a lesser extent producing artificial hybrids, in forest-tree genera. This has led to the amassing of an extensive literature which, until recently, has been largely of academic or of secondary practical interest. With the recent advent of a number of forest-tree breeding projects, however, these data have been automatically greatly increased in their scientific and practical importance. This fact is believed to warrant the present attempt to review some of the more readily available literature on forest-tree hybrids, and to summarize it primarily with the view of providing useful information to the forest-tree breeder.

Scope of the Work

Upon undertaking the work it soon became apparent that, under the circumstances of limited time and facilities, it would be necessary to restrict its scope. As a result the present paper includes only those genera of forest trees that are represented in North America by indigenous species of considerable economic importance. The available literature, which it is believed covered the subject fairly well, was examined for data on hybridization in genera meeting these requirements. It was found that some 405 hybrids were reported in 28 of these genera, as follows:

ABIES Link. Fir	3 hybrids	ARBUTUS L. Madrona	1 hybrid
ACER L. Maple	9 hybrids	BETULA L. Birch	13 hybrids
AESCULUS L. Buckeye	11 hybrids	CARYA Nutt. Hickory	7 hybrids
ALNUS L. Alder	8 hybrids	CASTANEA Adans. Chestnut	15 hybrids

CATALPA Scop. Catalpa	2 hybrids	PINUS Duham. Pine	20 hybrids
CORNUS L. Dogwood	5 hybrids	PLATANUS L. Plane-tree,	
CRATAEGUS L. Hawthorn	5 hybrids	Sycamore	1 hybrid
CUPRESSUS L. Cypress	1 hybrid	POPULUS L. Poplar	121 hybrids
GLEDITSIA L. Locust	1 hybrid	QUERCUS L. Oak	77 hybrids
ILEX L. Holly	3 hybrids	ROBINIA L. Locust	4 hybrids
JUGLANS L. Walnut	16 hybrids	SALIX L. Willow	42 hybrids
LARIX Adans. Larch	12 hybrids	TAXUS L. Yew	2 hybrids
MAGNOLIA L. Magnolia	5 hybrids	TILIA L. Basswood, Linden	8 hybrids
PICEA Dietr. Spruce	6 hybrids	TSUGA Carr. Hemlock	1 hybrid
		ULMUS L. Elm	6 hybrids

There are a number of genera of considerable economic importance in which it has not been possible to find reports of hybrids.   Among these genera are the following:

CARPINUS L. Hornbeam	LITHOCARPUS Blume Tan Bark Oak
CELTIS L. Hackberry	MORUS L. Mulberry
CHAMAECYPARIS Spach.	NYSSA L. Sour Gum, Cotton Gum
DIOSPYROS L. Persimmon	OSTRYA Scop. Hop Hornbeam, Ironwood
FAGUS L. Beech	PSEUDOTSUGA Carr. False Hemlock,
FRAXINUS L. Ash	Douglas Fir
GYMNOCLADUS L.	SASSAFRAS Nees. Sassafras
JUNIPERUS L. Juniper	SEQUOIA Endl. Redwood, Big Tree
LIBOCEDRUS Endl. Resin Cedar	SWIETENIA Jacq. Mahogany
LIQUIDAMBAR L. Sweet Gum	TAXODIUM Rich. Bald Cypress
LIRIODENDRON L. Yellow Poplar,	THUJA L. Arbor-vitae, Cedar
Tulip-tree	UMBELLULARIA Nutt.

Nomenclature

Any attempt to collect and summarize data involving botanical names applied by many authors and over many years is certain to be attended by considerable confusion arising from the inconsistencies of botanical nomenclature.   It is not within the author's field to bring order and completeness out of the disorder and incompleteness that exist in the literature covering the material dealt with.   For example, when a paper under review did not give authorities for the botanical names of parental material, no attempt was made to establish the exact identity of the material—the names of parents are given in the list without the addition of presumed authorities.   The Latin endings of certain specific names have been changed to give conformity with accepted usage.

To give a degree of consistency to the present paper, the International Rules are followed wherever possible.   For example, the sign × has been prefixed to all Latin names of hybrids, and the first letters of specific names are capitalized in cases where derivation is from the name of a person or of a genus.

Descriptive List of Forest-tree Hybrids

The summarization of collected data in forest-tree hybrids has been concerned primarily with giving, under selected headings, a highly condensed description which might prove useful to geneticists and plant breeders. When available, information on parental species has been included with the view of providing useful supplementary data. The descriptions are for the most part straightforward, but in some cases they may present difficulties. A short explanatory note on each heading follows:

No. The numbers under this heading are simply convenient numerical designations, which permit reference to any cross by number, and enable the reader to see at a glance the total number of crosses described for each genus.

Species involved. The practice of naming the female parent first is followed in general. Obviously, the rule cannot be applied very strictly in the case of natural hybrids of somewhat doubtful origin.

Nature of cross. The letters *N*, *A*, and *R* denote natural, artificial, and reciprocal crosses, respectively.

Chromosome nos. (n) involved. Under this heading are given the *n* (or reduced) chromosome numbers of the parental species involved. The sign × is placed between the numbers to correspond to its position under the heading *Species involved.* When two different numbers were reported in the literature one is given in parentheses—without, however, implying which number might be favoured by the author.

Name of hybrid. The sign × has been prefixed to all Latin names.

Notes on hybrid. Self-explanatory. In some cases space under this heading has been used for the extension of descriptions from other headings.

Country or region. Abbreviations are those in standard use.

Author and date of report or of origin. The author given may be the original reporter of the cross, the most important contributor to knowledge on the cross, or merely the author of the paper which chanced to be used as the main source in the present work. The reference number in parentheses refers to the main source in the present work. The date given before the semicolon refers to date of origin, the date after the author's name refers to the date of the report. The abbreviations *a.* and *b.* applied to date of origin denote *after* and *before*, respectively.

Other references. Here are listed by reference number the various papers which, in addition to the main source referred to in the preceding heading, have been used in compiling the information given for the cross in question.

No.	Species involved	Nature of cross	Chromosome nos. (n) involved	Name of hybrid
	***ABIES* LINK. FIR**			
1	A. Lowiana Murr. × A. grandis Lindl.	A		
2	A. cephalonica Loud. × A. pinsapo Boiss.	R	12 ×	× A. Vilmorinii Mast.
3	A. pinsapo Boiss. × A. Nordmanniana Spach.	N, R	× 12	× A. insignis Carr.
	***ACER* L. MAPLE**			
1	A. pseudoplatanus L. × A. monspessulanum L.		26 ×	× A. coriaceum Tausch
2	A. platanoides × A. laetum		13 ×	× A. Dieckii Pax.
3	A. platanoides L. × A. Lobelii Ten.	N	13 (11) ×	× A. Dieckii Pax.
4	A. campestre L. × A. monspessulanum L.	N		× A. Bornmuelleri Borb.
5	A. campestre L. × A. Lobelii Ten.			× A. zoeschense Pax.
6	A. opalus Mill. × A. monspessulanum L.			× A. Peronai Schwer.
7	A. opalus obtusatum Henry × A. monspessulanum L.	N		× A. velutinum Schwer.
8	A. tataricum L. × A. monspessulanum L.	N		× A. pusillum Schwer.
9	A. tataricum L. × A. pennsylvanicum L.	N		× A. Boscii Spach.
	***AESCULUS* L. BUCKEYE**			
1	A. Hippocastanum L. × A. Pavia L.	N	20 (19) × 20	× A. rubicunda Lodd. × A. carnea Willd.
2	A. glabra Willd. × A. hybrida DC.		20 ×	× A. arnoldiana Sarg.
3	A. discolor mollis Sarg. × A. neglecta georgiana Sarg.		20 × 20	× A. mutabilis (Spach.) Scheele
4	A. discolor mollis Sarg. × A. neglecta Lindl.		20 ×	× A. mutabilis induta Sarg.
5	A. discolor mollis Sarg. × A. glabra leucodermis Sarg.	N	20 × 20	× A. Bushii Schn.
6	A. neglecta Lindl. × A. Pavia L.	N	× 20	× A. Dupontii Sarg.
7	A. glabra Willd. × A. Pavia L.	N	20 × 20	× A. mississippiensis Sarg.
8	A. octandra Marsh. × A. Pavia L.		20 × 20	× A. octandra hybrida (DC.) Sarg.
9	A. discolor mollis Sarg. × A. neglecta georgiana Sarg.	N	20 × 20	× A. Harbisonii Sarg.
10	A. glabra Willd. × A. octandra Marsh.	N	20 ×	× A. marylandica Booth
11	A. neglecta georgiana Sarg. × A. Pavia L.	N	× 20	× A. Dupontii Hessei Sarg.
	***ALNUS* L. ALDER**			
1	A. cordata Desf. × A. subcordata C.A. Mey.	N	14 (21) × 14 (21)	
2	A. subcordata C.A. Mey. × A. incana Moench.		14 (21) × 14	× A. Koehneii Callier
3	A. cordata Desf. × A. glutinosa Gaertn.		14 (21) × 14	× A. elliptica Requiem.
4	A. glutinosa Gaertn. × A. incana Moench.	N, A	14 × 14	× A. hybrida A. Br.
5	A. japonica Sieb. & Zucc. × A. incana Moench.	N	14 × 14	× A. spectabilis Callier
6	A. subcordata C.A. Mey. × A. japonica Sieb. & Zucc.	N	14 × 14	× A. Spaethii Callier
7	A. rugosa Spring. × A. incana Moench.	N	14 × 14	× A. Aschersoniana Callier
8	A. rugosa Spring. × A. glutinosa Gaertn.	N	14 × 14	× A. silesiaca Fiek.
	***ARBUTUS* L. MADRONA**			
1	A. Andrachne L. × A. Unedo L.	N	13 ×	× A. andrachnoides Lk.
	***BETULA* L. BIRCH**			
1	B. pubescens Ehrh. × B. verrucosa Ehrh.	N, A	28 × 14	
		N, A	28 × 14	× B. Jackii Schn.
2	B. pumila Michx. × B. lenta L.			

Notes on hybrid	Country or region	Author and date of report or of origin	Other references	No.
intermediate; vigorous growth.	Denmark	1924; Larsen, 1937 (52)	51.	1
quantitative characters intermediate.	France	Flous, 1937 (23)	66, 71.	2
also × *A. Beissneriana* Mott., or *A. Ernesti* Rehd.	China, Eng., Fr.	1872; Rehder, 1927 (66)	71, 109.	3
also × *A. Duretti* Pax., *A. hybridum* Spach., *A. rotundilobum* Schwer.; cult. 1790.		Rehder, 1927 (66)	24, 27, 74, 82.	1
		Schreiner, 1937 (74)	24, 26, 18.	2
similar to *A. platanoides*.		Rehder, 1927 (66)	18, 26, 82.	3
	S.E. Europe	Rehder, 1927 (66)		4
cultivated 1880.		Rehder, 1927 (66)		5
cultivated 1905.	Italy, France	Rehder, 1927 (66)		6
cultivated 1894.		Rehder, 1927 (66)		7
cultivated 1870.		Rehder, 1927 (66)		8
		b. 1834; Rehder, 1927 (66)		9
fertile; habit, foliage like *A. Hip.*; flowers like *A. Pav.* F₁n=40	England	b. 1818; Crane, 1935 (17)	28, 43, 62, 66, 76, 84, 27.	1
	U.S.A., Mass.	1900; Rehder, 1927 (66)	27, 43, 74.	2
cultivated 1834.	U.S.A., Mass.	1900; Rehder, 1927 (66)	27, 43, 74.	3
also *A. mutabilis penduliflora* Sarg., cultivated 1834.	U.S.A., Mass.	1900; Rehder, 1927 (66)	27, 43, 74.	4
introduced U.S.A. 1901.	Ark., Miss., Mass.	Sargent, 1921 (67)	27, 43, 66, 74.	5
	U.S.A., Del.	a. 1820; Rehder, 1927 (66)	28, 74, 76.	6
intermediate; introduced U.S.A. 1913.	U.S.A., Miss.	Sargent, 1921 (67)	27, 28, 43, 66, 74. 76.	7
	Europe, U.S.A. (E)	Sargent, 1921 (67)	27, 28, 43, 66, 76.	8
introduced U.S.A. 1905; F₁n=20	U.S.A., Ga., Mass.	Sargent, 1921 (67)	27, 43, 66.	9
		Rehder, 1927 (66)	27, 43.	10
cultivated 1909.		Rehder, 1927 (66)	28, 76.	11
pronounced hybrid vigor.	Denmark	Larsen, 1937 (52)	27, 29, 46, 92, 93.	1
		Larsen, 1937 (52)	27, 29, 46, 66, 92, 93.	2
		Larsen, 1937 (52)	27, 29, 46, 66, 92, 93.	3
	Germany	Klotzsch, 1854 (52)	27, 29, 46, 66, 92, 93.	4
		b. 1908; Rehder, 1927 (66)	27, 93.	5
		b. 1908; Rehder, 1927 (66)	27, 93.	6
	Europe	Rehder, 1927 (66)	27, 29, 93, 98.	7
	Europe	Rehder, 1927 (66)	27, 29, 92, 93, 98.	8
intermediate.	Greece	1800; Rehder, 1927 (66)	27, 34.	1
fertile; intermediate; several forms.	N. Europe	Morgenthaler, 1915 (58)	27, 39, 52.	1
F₁n=21; tends to resemble *B. lenta* L.; cultivated 1895; shrub.	U.S.A. (N.E.)	Cousins, 1933 (16)	28, 66, 67, 97.	2

No.	Species involved	Nature of cross	Chromosome nos. (n) involved	Name of hybrid
	BETULA L. BIRCH (Continued)			
3	*B. coerulea-grandis* Blanch. × *B. populifolia* Ait.	N	× 14	× *B. coerulea* Blanch.
4	*B. papyrifera* Marsh. × *B. pumila* var. *glandulifera* Regel.	N	35 × 28	× *B. Sandbergii* Britt.
5	*B. lutea* Michx. × *B. pumila* var. *glandulifera* Regel.	N	42 × 28	× *B. Purpusii* Schn.
6	*B. nana* L. × *B. verrucosa* Ehrh.	N	14 × 14	× *B. intermedia* Thomas
7	*B. alaskana* Sarg. × *B. glandulosa* Michx.	N		× *B. commixta* Sarg.
8	*B. populifolia* Ait. × *B. papyrifera* Marsh.	N	14 (?) × 35	
9	*B. pendula* Roth. × *B. papyrifera* Marsh.	N	14 × 35	× *B. Koehnei* Schneid.
10	*B. pendula* Roth. × *B. pubescens* Ehrh.	N	14 ×	× *B. aurata* Bechst.
11	*B. pumila* L. × *B. papyrifera* Marsh.	N	28 × 35	× *B. excelsa* Ait.
12	*B. nana* L. × *B. pubescens* Ehrh.	N	14 ×	× *B. intermedia* Thomas
13	*B. nana* L. × *B. pendula* Roth.	N	14 × 14	× *B. fennica* Doerfl.
	CARYA Nutt. (**HICORIA** Rafn.) HICKORY			
1	*C. pecan* (Marsh.) Brit. × *C. cordiformis* (Wang.) Brit.	N	× 16	× *C. Brownii* (Sarg.) Ashe
2	*C. cordiformis* (Wang.) Brit. × *C. ovata* (Mill.) Brit.	N	16 × 16	× *C. Laneyi* (Sarg.) Sudw.
3	*C. pecan* (Marsh.) Brit. × *C. laciniosa* (Michx.) Sarg.	N	× 16	× *C. Nussbaumerii* (Sarg.) Sudw.
4	*C. alba* (L.) Brit. × *C. pecan* (Marsh.) Brit.	N	32 ×	× *C. Schneckii* (Sarg.) Sudw.
5	*C. laciniosa* Schn. × *H. ovata* K. Koch.	N	16 × 16	× *C. Dunbarii* Sarg.
6	*C. aquatica* Nutt. × *C. pecan* Engl. & Graebn.			× *C. texana* (Le Coute) C. DC.
7	*C. cordiformis* K. Koch. × *C. ovalis* Sarg.		16 × 32	× *C. Demareei* E. J. Palmer
	CASTANEA ADANS. CHESTNUT			
1	*C. mollissima* × *C. dentata*	A, R	× 12	
2	*C. crenata* (forest types) × *C. dentata*	A	11 × 12	
3	*C. crenata* (forest types) × *C. mollissima*	A	11 ×	
4	*C. crenata* (forest types) × *C. Henryi*	A	11 X	
5	*C. mollissima* × *C. Henryi*	A		
6	*C. pumila* × *C. Seguinii*	A		
7	*C. mollissima* × *C. Seguinii*	A		
8	*C. crenata* × *C. Seguinii*	A	11 ×	
9	(*C. crenata* × *C. dentata*) × *C. mollissima*	A, R		
10	(*C. mollissima* × *C. pumila*) × *C. dentata*	A	× 12	
11	*C. dentata* Borkh. × *C. pumila* Mill.	N	12 ×	× *C. neglecta* Dode.
12	*C. pumila* Mill. × *C. sativa* Mill.		× 11 (12)	
13	*C. sativa* Mill. × *C. dentata* Borkh.		11 (12) × 12	
14	*C. crenata* Sieb. & Zucc. × *C. dentata* Borkh.	A	11 × 12	
15	*C. crenata* Sieb. & Zucc. × *C. pumila* Mill.	N, A		

Notes on hybrid	Country or region	Author and date of report or of origin		Other references	No.
introduced 1905; $F_1 n = 14$.	N. Am.; N.S. to Vt.	Rehder, 1927	(66)	28, 97.	3
$F_1 n = 31$–32.	U.S.A., Minn.	Sargent, 1921	(67)	28, 29, 74, 97, 99.	4
$F_1 n = 45$; cultivated 1900; meiosis very abnormal.	U.S.A., Mich., Minn.	Sargent, 1921	(67)	28, 29, 66, 74, 97, 99.	5
		Schreiner, 1937	(74)	27, 28, 29, 39, 66, 94, 99.	6
	Canada, Yukon T.	Sargent, 1921	(67)		7
	U.S.A., Mass.	Rehder, 1927	(66)	28, 97.	8
meiosis *B. pen.* very abnormal; parents uncertain; cultivated 1905.		Rehder, 1927	(66)	28, 97.	9
meiosis *B pen.* very abnormal.	Europe	Rehder, 1927	(66)	28, 97.	10
hybridity uncertain; also *B. Borggreveana* Zabel; cultivated 1789.		Rehder, 1927	(66)	28, 29, 97, 99.	11
cultivated 1895.	Europe	Rehder, 1927	(66)	27, 28, 93, 94.	12
	Europe	Rehder, 1927	(66)	27, 28, 93, 94, 97.	13
similar to *C. pecan; var. variens* Sarg., cult.	U.S.A., Ark., Ohio	Sargent, 1921	(67)	29, 66, 74, 100.	1
$F_1 n = 16$, meiosis abnormal; var. *chateaugayensis* Sarg.; cultivated.	U.S.A., N.Y.; Can., Que., Ont.	Sargent, 1921	(67)	29, 66, 74, 100.	2
very vigorous; branch, fruit like *C. pecan;* leaves like *C. laciniosa;* cultivated.	U.S.A., Ill., Ind., Ia., Mo.	Sargent, 1921	(67)	29, 66, 74, 100, 106.	3
parentage not certain; cultivated.	U.S.A., Ill., Ia.	Sargent, 1921	(67)	29, 66, 74, 100.	4
intermediate, but parentage not certain.	U.S.A., Golah, N.Y.	Sargent, 1921	(67)	29, 100.	5
range of variability in fruit characters between *C. aquatica* and *C. pecan.*	U.S.A. (S.W.)	Palmer, 1937	(108)		6
intermediate in foliage, fruit and winter buds.	U.S.A., Ark.	Palmer, 1937	(108)	29, 100.	7
marked hybrid vigor; 272 seedlings.	U.S.A., N.Y., etc.	Schreiner, 1937	(73)	29, 46.	1
backcrosses; F_2 produced; timber types; blight resistant.	U.S.A., N.Y., etc.	Schreiner, 1937	(73)	27, 29, 46, 93.	2
hybrid vigor, 44 seedlings.	U.S.A.	Schreiner, 1937	(73)	27, 93.	3
hybrid vigor, 3 seedlings.	U.S.A.	Schreiner, 1937	(73)	27, 93.	4
hybrid vigor, 16 seedlings.	U.S.A.	Schreiner, 1937	(73)		5
everblooming of *C. Seguinii* dominant; 21 seedlings.	U.S.A.	Schreiner, 1937	(73)		6
everblooming of *C. Seguinii* dominant; 19 seedlings.	U.S.A.	Schreiner, 1937	(73)		7
everblooming of *C. Seguinii* dominant; 24 seedlings.	U.S.A.	Schreiner, 1937	(73)	27, 93.	8
	U.S.A., N.Y.	Schreiner, 1937	(73)		9
	U.S.A., N.Y.	Schreiner, 1937	(73)	29, 46.	10
intermediate.	Macon Co., N.C., U.S.A.	Sargent, 1921	(67)	29, 46, 66.	11
fertile.	U.S.A., N.J.	1903; Van Fleet; Fairchild, 1918.	(21)	27, 29, 46, 66, 93.	12
		Rehder, 1927	(66)	27, 29, 46, 93.	13
variable dominance; F_2 produced; remarkable vigor.	U.S.A., Ill.	1908; Detlefsen & Ruth, 1922.	(19)	27, 29, 46, 66, 93.	14
blight resistance (from *C. crenata*) dominant.		b. 1911; Rehder, 1927	(66)	6, 27, 93.	15

No.	Species involved	Nature of cross	Chromosome nos. (n) involved	Name of hybrid
	CATALPA SCOP. CATALPA			
1	*C. bignonioides* Walt. × *C. ovata* Don			× *C. hybrida* Spaeth
2	*C. Kaempferi* (= *C. ovata*) × *C. bignonioides*			
	CORNUS L. DOGWOOD			
1	*C. rugosa* Lam. × *C. stolonifera* Michx.	N		× *C. Slavinii* Rehd.
2	*C. obliqua* Raf. × *C. racemosa* Lam.	N		× *C. arnoldiana* Rehd.
3	*C. macrophylla* Wall. × *C. Amomum* Mill.	N		× *C. Horseyi* Rehd.
4	*C. macrophylla* Wall. × *C. asperifolia* Michx.	N		× *C. Dunbarii* Rehd.
5	*C. paucinervis* Hance × *C. Amomum* Mill.	N		× *C. dubia* Rehd.
	CRATAEGUS L. HAWTHORN			
1	*C. oxyacantha* L. × *C. monogyna* Jacq.	N	16 × 16	× *C. media* Bechst.
2	*C. crus-galli* L. × *C. pubescens* Steud.	N	24 ×	× *C. Lavallei* Herincq
3	*C. sanguinea* Pall. × *C. nigra* Kit.	N	16 (?) ×	× *C. Lambertiana* Lge.
4	*C. pentagyna* Waldst. & Kit. × *C. crus-galli* L.	N	× 24	× *C. hiemalis* Lge.
5	*C. tanacetifolia* Pers. × *C. punctata.*	N		× *C. Dippeliana* Lge.
	CUPRESSUS L. CYPRESS			
1	*C. macrocarpa* Hartw. × *C. nootkatensis* Don	N, R		× *C. Leylandii* J. & D.
	GLEDITSIA L. LOCUST			
1	*G. triacanthos* L. × *G. aquatica* Marsh.			× *G. texana* Sarg.
	ILEX L. HOLLY			
1	*I. Aquifolium* L. × *I. perado*			× *I. altaclarensis* Dallim.
2	*I. Aquifolium* L. × *I. latifolia* Thunb.			× *I. Koehneana* Loes.
3	*I. Aquifolium* L. × *I. dipyrena* Wall.			× *I. Beanii* Rehd.
	JUGLANS L. WALNUT			
1	*J. regia* L. × *J. nigra* L.	N, R	16 × 16	× *J. intermedia* Carr.
2	*J. regia* L. × *J. cinerea* L.	·N	16 × 16	× *J. quadrangulata* Rehd.
3	*J. nigra* L. × *J. Hindsii* Rehd.	A	16 × 17	Royal Black Walnut
4	*J. Hindsii* Rehd. × *J. regia* L.	A	17 × 16	Paradox Walnut
5	*J. Sieboldiana* Max. × *J. cinerea* L.	N, A	16 × 16	× *J. Bixbyi* Rehd.
6	*J. Sieboldiana* Max. × *J. regia* L.		16 × 16	× *J. notha* Rehd.
7	*J. Hindsii* × Royal hybrid	A, R	17 ×	
8	Royal hybrid × *J. mandshurica*	A	× 16	
9	Royal hybrid × *J. regia*	A	× 16	
10	*J. mandshurica* × *J. regia*	A	16 × 16	
11	*J. mandshurica* × *J. Sieboldiana*	A	16 × 16?	
12	*J. mandshurica* × *J. cordiformis*	A	16 × 16?	
13	*J. mandshurica* × *J. nigra*	A	16 × 16	
14	*J. mandshurica* × *J. cinerea*	A, R	16 × 16	
15	*J. nigra* × *J. cinerea*		16 × 16	
16	*J. Sieboldiana* × *J. nigra*		16(?) × 16	

Notes on hybrid	Country or region	Author and date of report or of origin	Other references	No.
intermediate, two varieties.		about 1874; Rehder, (66) 1927		1
characteristics of both parents; increased vigor, hardiness.	U.S.A., Ind., Mo.	Jones & Filley, 1920 (105)		2
cultivated.		b. 1910; Rehder, 1927 (66)		1
cultivated.		b. 1900; Rehder, 1927 (66)		2
cultivated.		b. 1919; Rehder, 1927 (66)		3
cultivated.		b. 1919; Rehder, 1927 (66)		4
cultivated.		b. 1920; Rehder, 1927 (66)		5
fertile.	Denmark	Raunkiaer, 1925; (52) Larsen, 1937	26, 55, 66.	1
parentage not certain.		b. 1880; Rehder, 1927 (66)	26, 55.	2
cultivated 1871; parentage not certain.		Rehder, 1927 (66)	26, 55.	3
parentage not certain.		b. 1880; Rehder, 1927 (66)	26, 55.	4
parentage not certain.		about 1830; Rehder, (66) 1927		5
intermediate in cone and seed characters.	England	Jackson & Dallimore, (104) 1926		1
	U.S.A., Brazoria, Texas	Rehder, 1927 (66)	67.	1
cultivated since 1838.		Rehder, 1927 (66)		1
cultivated since 1900.		Rehder, 1927 (66)		2
cultivated since 1900.		Rehder, 1927 (66)		3
resembles *J. regia*; very vigorous; largest walnut tree.	Europe; U.S.A.	1863; Larsen, 1937 (52)	29, 65, 66, 67, 75, 100.	1
resembles *J. regia*.	Europe; U.S.A.	Sargent, 1921 (67)	29, 52, 66, 100.	2
100 ft. high, 9 ft. in circumference in 16 years.	U.S.A., Cal.	1877; Burbank (52)	27, 29, 64, 66, 67, 73, 100, 106.	3
80 ft. high, 6 ft. in circumference in 15 years.	U.S.A., Cal.	1887; Burbank (52)	27, 29, 64, 66, 100, 106.	4
intermediate; definite hybrid vigor.	Denmark; U.S.A.	1903; 1929; Rehder, (66) 1927	29, 51, 52, 65, 67, 100, 101.	5
nut resembles *J. Sieboldiana*; meiosis very irregular.		1878; Rehder, 1927 (66)	29, 65, 100.	6
represents a backcross (see No. 3).	U.S.A., Cal.	Schreiner, 1937 (73)	27, 64.	7
	U.S.A., Cal.	Schreiner, 1937 (73)	29, 100.	8
	U.S.A., Cal.	Schreiner, 1937 (73)	29, 100.	9
J. mandshurica characters dominant; greater vigor than *J. mandshurica*.	Russia .	Yablokov, 1936 (101)	29, 100.	10
variable; winter hardy.	Russia	Yablokov, 1936 (101)	29, 100.	11
	Russia	Yablokov, 1936 (101)	29, 100.	12
	Russia	Yablokov, 1936 (101)	29, 100.	13
	Russia	Yablokov, 1936 (101)	29, 100.	14
	U.S.A.; East	Reed, 1936 (65)	29, 100.	15
	U.S.A.; East	Reed, 1936 (65)	29, 100.	16

No.	Species involved	Nature of cross	Chromosome nos. (n) involved	Name of hybrid
	LARIX ADANS. LARCH			
1	*L. Gmelini* Gord. × *L. sibirica* Ledeb.	N		× *L. Czekanowskii* Szafer
2	*L. Kaempferi* Sarg. × *L. decidua* Mill.	N, A	12 × 12	× *L. eurolepis* Henry (Dunkeld larch)
3	*L. Gmelini* Pilg. × *L. Kaempferi* Sarg.	N, A	× 12	
4	*L. decidua* Mill. × *L. Kaempferi* Sarg.	A	12 × 12	
5	*L. decidua* Mill. × *L. laricina* K. Koch	A	12 × 12	
6	*L. decidua* Mill. × *L. occidentalis* Nutt.	A	12 × 12	
7	*L. Kaempferi* Sarg. × *L. Gmelini olgensis* Ostf. & Lars.	N, A	12 ×	
8	*L. laricina* K. Koch × *L. decidua* Mill.	A	× 12	× *L. pendula* Salisb.
9	*L. leptolepis* × *L. europaea* (syn. with cross 2?)	?		Stabrand
10	*L. decidua* Mill. × *L. leptolepis* Murr. (syn. with cross 4?)		12 ×	
11	*L. occidentalis* Nutt. × *L. Lyallii*	N	12 ×	
12	*L. Potaninii* × *L. Mastersiana*	N		
	MAGNOLIA L. MAGNOLIA			
1	*M. virginiana* L. × *M. tripetala* L.	N	19 × 45	× *M. major* or *Thompsoniana* Sarg.
2	*M. denudata* Desr. × *M. Campbellii*		48 (57) ×	× *M. Veitchii* Bean
3	*M. stellata* Maxim. × *M. kobus* Thunb.			× *M. Loebneri* Kache
4	*M. denudata* Desr. × *M. liliflora* Desr.		48 (57) × 38	× *M. Soulangeana* Soul.
5	*M. denudata* × *M. liliflora*		57 × 38	× *M. purpurascens*
	PICEA DIETR. SPRUCE			
1	*P. sitchensis* Carr. × *P. canadensis* B.S.P.	N, A		
2	*P. Engelmanni* Englm. × *P. sitchensis* Carr.			
3	*P. Engelmanni* Englm. × *P. canadensis* B.S.P.			
4	*P. rubra* Link. × *P. excelsa* Link.	A		
5	*P. mariana* B.S.P. × *P. jezoensis* Maxim.	R	12 ×	× *P. moseri* Mast.
6	*P. Glehnii* (Fr. Schm.) Mast. × *P. jezoensis* var. *hondoensis* (Mayr) Rehd.	N		× *P. notha* Rehd.
	PINUS DUHAM. PINE			
1	*P. mugo* Turra. × *P. sylvestris* L.	N	× 12	× *P. rhaetica* Bruegg.
2	*P. nigra* Arnold × *P. sylvestris* L.	N	12 × 12	× *P. neilreichiana*
3	*P. sylvestris* L. × *P. nigricans*	A	12 ×	
4	*P. montana* Mill. × *P. sylvestris* L.	N .		× *P. rhaetica*
5	*P. palustris* × *P. taeda*	N		× *P. Sondereggeri*
6	*P. rigida* × *P. echinata*	N	× 12	
7	*P. Murrayana* × *P. Banksiana*	N	× 12	
8	*P. halepensis* × *P. pinaster*	N		
9	*P. nigra* × *P. densiflora*	A	12 ×	
10	*P. sylvestris* × *P. nigra*	A	× 12	
11	*P. montana* × *P. nigra*	N	× 12	× *P. Wettsteiniana*
12	*P. attenuata* × *P. radiata*	A		
13	*P. ayacahuite* Ehrenb. × *P. excelsa* Wall.	N		× *P. Holfordiana* Jacks.
14	*P. palustris* × *P. caribaea*	A		

Notes on hybrid	Country or region	Author and date of report or of origin	Other references	No.
fertile, intermediate.	Russia, E. Siberia	Szafer; Sukatschew (52)	53.	1
fertile, intermediate; extraordinarily vigorous growth.	England, Scotland, Denmark	1900; Henry & Flood, 1919 (40)	51, 52, 53, 66, 71, 73.	2
fertile; "large, handsome."	Denmark	Larsen, 1900 (52)	51, 53, 71, 73.	3
	Denmark; U.S.A.	Larsen, 1937 (52)	53, 71.	4
	Denmark; U.S.A.	Larsen, 1937 (52)	53, 71	5
F_1 triploid (n = 18); vigorous, robust growth.	Denmark, U.S.A.	Larsen, 1937 (52)	53, 71.	6
	Denmark, U.S.A.	Larsen, 1937 (52)	53, 71.	7
resembles *L. laricina*.	Denmark, U.S.A.	Larsen, 1937 (52)	53, 66, 71.	8
L. leptolepis dominant in hardiness, vigor; compares favorably with Dunkeld larch.	Denmark	Pedersen, 1933 (61)	73.	9
	England	Anonymous, 1935 (2)	71.	10
hybridity probable.		Ostenfeld & Larsen, 1930 (60)	53.	11
hybridity probable.		Ostenfeld & Larsen, 1930 (60)	53.	12
intermediate.	England; U.S.A.	1808; Sargent, 1921 (67)	22, 27, 56, 66.	1
		1907; Rehder, 1927 (66)	1, 27, 87, 103.	2
"doubtful".		b. 1910; Rehder, 1927 (66)		3
		1820; Rehder, 1927 (66)	1, 27, 87, 103.	4
$F_1 2n = 95$.	Japan	Yasui, K., 1937 (103)		5
pronounced hybrid **vigor**.	N. Europe, especially Denmark	Fabricius, 1926 (52)	51, 73.	1
	Denmark	1934; Larsen, 1937 (52)	51.	2
	Denmark	1934; Larsen, 1937 (52)	51, 73.	3
	U.S.A., N.Y.	1932; Heimburger (73)	47.	4
		Rehder, 1927 (66)	71.	5
clearly intermediate.	U.S.A., Mass.	1894; Rehder, 1939 (109)		6
fertile; intermediate; several forms.	Germany	Beissner, 1909; Petersen (52)	66, 71.	1
fertile.	Europe	Larsen, 1937 (52)	4, 71, 73.	2
see No. 10.	Germany	Klotzsch, 1854 (52)	71.	3
artificial crosses failed.	Denmark. (N. Jutland)	Larsen, 1934 (51)	3, 4, 73.	4
intermediate.	U.S.A., La.	Chapman, 1924 (14)	3, 4, 73.	5
	U.S.A., Pa.	Perry (4)	71.	6
	Canada, N. Alta.	Holman (4)	71.	7
	U.S.A.	Austin, 1929 (4)	3, 71.	8
	U.S.A.	1914; Blakeslee (4)	3, 71.	9
see No. 3	Germany	1845; Klotzsch (4)	3, 71.	10
	Germany	Austin, 1929 (4)	3, 71, 73.	11
hardiness of *P. attenuata;* vigor of *P. radiata;* F_1 produced.	U.S.A., Cal.	Austin, 1937 (5)	73.	12
seed and cone characters intermediate in general.	England	Jackson, 1933 (45)		13
types resistant to brown spot.	U.S.A., New Orleans, La.	Schreiner, 1937 (73)		14

No.	Species involved	Nature of cross	Chromosome nos. (n) involved	Name of hybrid
	PINUS DUHAM. PINE (Continued)			
15	P. Sondereggeri × P. pålustris	A		
16	P. Sondereggeri × P. taeda	A		
17	P. caribaea × P. taeda	N		
18	P. echinata × P. taeda	N	12 ×	
19	P. ponderosa Jeffreyi × P. ponderosa		× 12	
20	P. rigida × P. taeda	N	12 ×	
	PLATANUS L. PLANE-TREE, SYCAMORE			
1	P. orientalis L. × P. occidentalis L.	N	10-11 (8) × 10-11 (8)	× P. acerifolia Willd.
	POPULUS L. POPLAR			
1	P. alba L. × P. tremula L.	N, A, R	19 × 19 (4)	× P. canescens Sm.
2	P. balsamifera L. × P. nigra L.	N	19 (38) × 19	× P. canadensis Moench.
3	P. laurifolia Ledeb. × P. nigra var. italica Dur.	N	× 19	× P. berolinensis Dippel.
4	P. angulata Ait. × P. nigra var. plantierensis Schneid.	N, A	× 19	× P. robusta Schneid.
5	P. angulata Ait. × P. trichocarpa Torr. & Gray	A, R	× 19	× P. generosa Henry
6	P. pyramidalis × P. nigra	N	× 19	× P. charkowiensis
7	P. deltoides var. monilifera × P. nigra var. typica	N	× 19	× P. serotina
8	P. nigra × P. serotina	N	19 × 19	× P. regenerata
9	× P. regenerata × P. nigra var. italica	N	× 19	× P. Eugenei
10	P. balsamifera virginiana × P. grandidentata	A	× 19	
11	P. acuminata Rydb. × P. Sargentii Dode.	N, R		× P. Andrewsii Sarg.
12	P. Fremontii S. Wats. × P. trichocarpa Hook.	N	× 19	× P. Parryi Sarg.
13	P. balsamifera virginiana Sarg. × P. tacamahaca Mill.	N		× P. Jackii Sarg.
14	× P. canescens Sm. × P. tremula L.	A	19 × 19 (4)	
15	P. angulata Späth × P. canadensis		× 4	× P. eucalyptus
16	P. canadensis × P. pyramidalis		4 ×	
17	P. canescens Sm. × P. tremuloides Michx.	A	19 × 19	
18	P. laurifolia Ledeb. × P. balsamifera L. (?)	N		× P. Petrowskyana Schneid.
19	P. laurifolia Ledeb. × P. nigra L.	N		× P. Rasumowskyana Schneid.
20	P. laurifolia Ledeb. × P. tristis Fisch.	N		× P. Wobstii Schroed.
21	P. alba L. × P. tremuloides Michx.	A	19 (?) × 19	
22	P. alba L. × P. grandidentata Michx.	A, N	19 (?) × 19	
23	P. canescens Sm. × P. grandidentata Michx.	A	19 (?) × 19	
24	P. tremuloides Michx. × P. grandidentata Michx.	A	19 × 19	
25	P. alba × P. alba nivea	A	19 × 19	
26	P. alba × P. canescens	A	19 × 19	
27	P. alba × P. adenopada	A	19 ×	

Notes on hybrid	Country or region	Author and date of report or of origin	Other references	No.
backcross.	U.S.A., New Orleans	Schreiner, 1937 (73)		15
backcross.	U.S.A., New Orleans	Schreiner, 1937 (73)		16
hybridity probable; ten trees.	U.S.A., Cal.	Schreiner, 1937 (73)		17
vigor exceeds *P. echinata*; hybridity probable; 14 trees.	U.S.A., Cal.	Schreiner, 1937 (73)	71.	18
vigor greatly exceeds *P. p. Jeffreyi*; hybridity certain.	U.S.A., Cal.	Schreiner, 1937 (73)	71.	19
hybridity probable, four trees.	U.S.A., Cal.	Schreiner, 1937 (73)	71.	20
fertile; London plane-tree; hardy; $F_1n = 10-11$ (21).	Eng.; Europe generally	b. 1700; Larsen, 1937 (52)	11, 12, 27, 66, 70, 96.	1
fertile; most vigor where *P. alba* is female; $F_1n = 19$.	Central Europe; U.S.A.	Wettstein, 1934 (89)	9, 27, 30, 33, 52, 63, 78, 90.	1
fertile; also var. *Eugenei* Schelle; $F_1n = 4$.	Europe, N. Amer.	Rehder, 1927 (66)	9, 26, 27, 33, 52, 67.	2
fertile; hardy, N.W. prairies; original female from Germany.	Europe	b. 1870; Dippel, 1892 (13)	9, 26, 52, 66.	3
male only; very rapid growth.	France; U.S.A.	1895; Schneider, 1904 (13)	9, 26, 52, 66, 78.	4
very rapid growth; $F_1n = 19$.	England, U.S.A.	1900, 1912; Henry, 1914 (13)	8, 26, 27, 52, 57, 66, 78.	5
intermediate; rapid growth, hardy	Russia (Charkow)	Kucera, 1902 (13)	9, 26.	6
male only; very rapid growth; frost hardy $F_1n = 19$.	France	Duhamel, 1755 (13)	8, 9, 26, 27.	7
female only; leaves and branches as *P. ser.*; very rapid growth.	France (Arcueil)	1814; Henry, 1913 (13)	8, 9, 26, 27.	8
male only; $F_1n = 19$, canker susceptible.	France (Metz)	1832; Schneider, 1904 (13)	8, 9, 26, 27, 63.	9
P. grand. dominant in nearly all characters; vigor very variable.	U.S.A. (N. East)	Stout & Schreiner, 1934 (79)	9, 26, 63, 78.	10
intermediate; introduced U.S.A. 1913.	U.S.A., Colorado	Sargent, 1921 (67)	66.	11
intermediate.	U.S.A., Cal.	Sargent, 1921 (67)	26, 57.	12
intermediate; introduced U.S.A. 1900; cultivated occasionally.	U S.A. Mich., Vt.; Can., Que.	Sargent, 1921 (67)	66.	13
less vigor than *P. canescens*; backcross.	Germany	Wettstein, 1937 (90)	9, 26, 27, 33, 63.	14
more vigor than *P. can.*; resistant to *Melampsora populini* in F_2.	Germany	Wettstein, 1937 (90)	27, 33, 91.	15
P. pyramidalis dominant.	Germany	Wettstein, 1937 (90)	27, 33.	16
F_1 died as small seedling.	Canada, Ont.	Heimburger, 1936 (38)	20, 27, 63, 73.	17
P. laur. as parent doubtful.	U.S.A.; Canada	b. 1882; Rehder, 1927 (66)		18
P. laur. as parent doubtful.	U.S.A.; Canada	b. 1882; Rehder, 1927 (66)		19
similar to *P. laurifolia.*	U.S.A.	Rehder, 1927 (66)		20
F_1 died as small seedling.	Canada, Ont.	Heimburger, 1936 (38)	20, 27, 47, 63.	21
intermediate.	Ont., Canada	Heimburger, 1936 (38)	47, 63.	22
characters very variable.	Ont., Canada	Heimburger, 1936 (38)	63.	23
intermediate.	Ont., Canada	Heimburger, 1936 (38)	20, 27, 63.	24
67 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933 (78)	63.	25
8 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933 (78)	63.	26
34 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933 (78)	63.	27

No.	Species involved	Nature of cross	Chromosome nos. (n) involved	Name of hybrid
	***POPULUS* L.** POPLAR (Continued)			
28	*P. alba* × *P. tremula Davidiana*	A	19 ×	
29	*P. tremuloides* × *P. tremula*	A	19 × 19	
30	*P. angulata* × *P. balsamifera virginiana*	A		
31	*P. angulata* × Cottonwood (unidentified)	A		
32	*P. angulata* × *P. caudina*	A		
33	*P. angulata* × *P. incrassata*	A		
34	*P. angulata* × clone Robusta	A		
35	*P. angulata* × clone Volga	A		
36	*P. angulata* × *P. berolinensis*	A		
37	*P. angulata* × *P. trichocarpa*	A	× 19	
38	*P. balsamifera virginiana* × Cottonwood (unidentified)	A		
39	*P. balsamifera virginiana* × *P. caudina*	A		
40	*P. balsamifera virginiana* × *P. incrassata*	A		
41	*P. balsamifera virginiana* × *P. nigra plantierensis*	A		
42	*P. balsamifera virginiana* × clone Robusta	A		
43	*P. balsamifera virginiana* × clone Volga	A		
44	*P. balsamifera virginiana* × *P. berolinensis*	A		
45	*P. balsamifera virginiana* × *P. trichocarpa*	A	× 19	
46	*P. charkowiensis* × *P. balsamifera virginiana*	A		
47	*P. charkowiensis* × Cottonwood (unidentified)	A		
48	*P. charkowiensis* × *P. caudina*	A		
49	*P. charkowiensis* × *P. incrassata*	A		
50	*P. charkowiensis* × *P. nigra plantierensis*	A		
51	*P. charkowiensis* × clone Robusta	A		
52	*P. charkowiensis* × clone Volga	A		
53	*P. charkowiensis* × *P. berolinensis*	A		
54	*P. charkowiensis* × *P. trichocarpa*	A	× 19	
55	*P. Fremontii* × *P. balsamifera virginiana*	A		
56	*P. Fremontii* × Cottonwood (unidentified)	A		
57	*P. Fremontii* × *P. incrassata*	A		

Notes on hybrid	Country or region	Author and date of report or of origin		Other references	No.
16 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	63.	28
11 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	9, 20, 27, 63.	29
583 seedlings; susceptible to rust.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		30
248 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		31
99 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		32
203 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		33
60 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		34
214 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		35
205 seedlings, 2 very promising.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		36
264 seedlings, 1 very promising. (See No. 5).	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	26, 57.	37
18 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		38
189 seedlings, 1 very promising.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		39
208 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		40
183 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		41 '
7 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		42
216 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		43
245 seedlings, 3 very promising.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		44
705 seedlings, 3 very promising.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	26, 57.	45
288 seedlings, 4 very promising.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		46
267 seedlings, 2 very promising.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		47
266 seedlings, 5 very promising.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		48
263 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		49
312 seedlings, 2 very promising; pyramidal.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		50
52 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		51
188 seedlings; pyramidal.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		52
249 seedlings, 3 very promising; pyramidal.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		53
221 seedlings, 2 very promising.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	26, 57.	54
7 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		55
9 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		56
108 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		57

No.	Species involved	Nature of cross	Chromosome nos. (n) involved	Name of hybrid
	POPULUS L. POPLAR (Continued)			
58	P. Fremontii × P. nigra plantierensis	A		
59	P. Fremontii × clone Volga	A		
60	P. Fremontii × P. berolinensis	A		
61	P. Fremontii × P. trichocarpa	A	× 19	
62	P. nigra × clone Eugenei	A	19 × 19	
63	P. nigra × P. nigra Italica (clone Lombardy)	A	19 ×	
64	P. nigra × P. berolinensis Rossica	A	19 ×	
65	P. nigra × P. laurifolia	A	19 ×	Frye, Rumford, Strathglass
66	P. nigra × P. Simonii	A	19 × 38	
67	P. nigra × P. trichocarpa	A	19 × 19	Roxbury
68	P. nigra baatanicorum vitrum × P. balsamifera virginiana	A		
69	P. nigra baatanicorum vitrum × Cottonwood	A		
70	P. nigra baatanicorum vitrum × P. caudina	A		
71	P. nigra baatanicorum vitrum × P. incrassata	A		
72	P. nigra baatanicorum vitrum × P. nigra plantierensis	A		
73	P. nigra baatanicorum vitrum × clone Volga	A		
74	P. nigra baatanicorum vitrum × P. trichocarpa	A	× 19	
75	P. nigra betulifolia × P. balsamifera virginiana	A		
76	P. nigra betulifolia × Cottonwood	A		
77	P. nigra betulifolia × P. incrassata	A		
78	P. nigra betulifolia × P. nigra plantierensis	A		
79	P. nigra betulifolia × clone Volga	A		
80	P. nigra betulifolia × P. trichocarpa	A	× 19	Andover
81	P. Sargentii × P. balsamifera virginiana	A		
82	P. Sargentii × clone Eugenei	A	× 19	
83	P. Sargentii × P. nigra Italica (clone Lombardy)	A		
84	P. Sargentii × P. berolinensis	A		
85	P. Sargentii × P. berolinensis Rossica	A		
86	P. Sargentii × P. laurifolia	A		
87	P. Sargentii × P. Simonii	A	× 38	

Notes on hybrid	Country or region	Author and date of report or of origin		Other references	No.
194 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		58
317 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		59
69 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		60
125 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	26, 57.	61
49 seedlings; pyramidal.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	8, 9, 26, 27, 63.	62
44 seedlings; pyramidal.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	9, 26.	63
217 seedlings, 2 very promising.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	9, 26.	64
377 seedlings, 10 very promising.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	9, 26, 80.	65
2 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	9, 26, 57.	66
200 seedlings; 3 very promising; remarkable vigor.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	9, 26, 57, 80.	67
6 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		68
60 seedlings; pyramidal.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		69
51 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		70
10 seedlings; pyramidal.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		71
157 seedlings; pyramidal.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		72
170 seedlings; pyramidal.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		73
121 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	26, 57.	74
11 seedlings; pyramidal.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		75
11 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		76
141 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		77
65 seedlings; pyramidal.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		78
166 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		79
209 seedlings, 1 very promising; remarkable vigor; pyramidal.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	26, 57, 80.	80
72 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		81
50 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	8, 27, 63.	82
25 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		83
149 seedlings, 2 very promising.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		84
309 seedlings, 2 very promising.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		85
51 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		86
14 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	26, 57.	87

No.	Species involved	Nature of cross	Chromosome nos. (n) involved	Name of hybrid
	POPULUS L. POPLAR (Continued)			
88	P. Sargentii × P. trichocarpa	A	× 19	
89	P. berolinensis × P. caudina	A		
90	P. berolinensis × P. nigra plantierensis	A		
91	P. berolinensis × clone Serotina	A	× 19	
92	P. berolinensis × clone Volga	A		
93	P. berolinensis × P. trichocarpa	A	× 19	
94	P. Maximowiczii × P. caudina	A		
95	P. Maximowiczii × P. incrassata	A		
96	P. Maximowiczii × P. nigra plantierensis	A		Rochester
97	P. Maximowiczii × P. berolinensis	A		Geneva, Oxford
98	P. Maximowiczii × P. trichocarpa	A	× 19	Androscoggin
99	P. Petrowskyana × P. caudina	A		
100	P. Rasumowskyana × P. grandidentata	A	× 19	
101	P. Rasumowskyana × P. balsamifera virginiana	A		
102	P Rasumowskyana × Cottonwood (unidentified)	A		
103	P. Rasumowskyana × P. caudina	A		
104	P. Rasumowskyana × P. incrassata	A		
105	P Rasumowskyana × P. nigra plantierensis	A		
106	P. Rasumowskyana × clone Volga	A		
107	P. Rasumowskyana × P. berolinensis	A		
108	P. Rasumowskyana × P. trichocarpa	A	× 19	
109	P. Simonii × P. grandidentata	A	38 × 19	
110	P. Simonii × P. caudina	A	38 ×	
111	P Simonii × P. incrassata	A	38 ×	
112	P. Simonii × P. nigra plantierensis	A	38 ×	
113	P. Simonii × clone Robusta	A	38 ×	
114	P. Simonii × clone Volga	A	38 ×	
115	P. Simonii × P. berolinensis	A	38 ×	
116	P. Simonii × P trichocarpa	A	38 × 19	

Notes on hybrid	Country or region	Author and date of report or of origin		Other references	No.
233 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	26, 57.	88
8 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		89
17 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		90
29 seedlings	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	8, 27.	91
62 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		92
27 seedlings, 2 very promising.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	26, 57	93
179 seedlings, 1 very promising.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		94
2 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		95
145 seedlings, 1 very promising.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	80.	96
112 seedlings; very vigorous; rust resistant; late, fall growth; 8 very prominent.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	80.	97
5 seedlings, 3 very promising.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	26, 57, 80.	98
25 seedlings, 1 very promising.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		99
2 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	63.	100
56 seedlings.	U.S.A (N. East)	Stout & Schreiner, 1933	(78)		101
30 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		102
70 seedlings, 1 very promising.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		103
25 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		104
76 seedlings; half of the hybrids are columnar.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		105
81 seedlings, 1 very promising.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		106
183 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)		107
148 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	26, 57.	108
32 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	26, 57, 63.	109
99 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	26, 57.	110
75 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	26, 57.	111
176 seedlings; pyramidal.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	26, 57.	112
1 seedling.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	26, 57.	113
155 seedlings; pyramidal.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	26, 57.	114
189 seedlings, 1 very promising; columnar to spreading.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	26, 57.	115
44 seedlings.	U.S.A. (N. East)	Stout & Schreiner, 1933	(78)	26, 57.	116

No.	Species involved	Nature of cross	Chromosome nos. (n) involved	Name of hybrid
	POPULUS L. POPLAR (Continued)			
117	P. tacamahaca candicans (clone Balm of Gilead) × P. balsamifera virginiana	A		
118	P. tacamahaca candicans × P. nigra plantierensis	A		
119	P. tacamahaca candicans × P. berolinensis	A		Maine
120	P. tacamahaca candicans × P. laurifolia	A		
121	P. nigra × P. deltoidea var. missouriensis	N	19 ×	Carolina poplar
	QUERCUS L. OAK			
1	Q. robur L. × Q. sessiliflora Salisb.	N, A	12 (11) × 12 (11)	× Q. rosacea Bechst.
2	Q. sessiliflora Martyn. × Q. robur L.		12 × 12	
3	Q. Cerris L. × Q. suber L.	N	12 (11) × 12	× Q. hispanica Lam.
4	Q. robur Mill. × Q. pedunculata (Q. robur L.)	A	× 12 (11)	
5	Q. Ilex L. × Q. suber P. Cout.		12 × 12	
6	Q. coccifera L. × Q. Ilex L.		12 × 12	
7	Q. robur L. (pedunculata) × Q. macrocarpa		12 (11) × 12 (6)	
8	Q. Ilex L. × Q. sessilis Ehrh. (sessiliflora Salisb.)	·	12 × 11 ﹍	× Q. Koehnii
9	Q. glaucophylla Seemen × Q. clivicola Trel. & C. H. Muell.	N		× Q. pastorensis C. H. Muell.
10	Q. breviloba (Torr.) Sarg. × Q. stellata Wang.	N		× Q. Mahoni E. J. Palmer
11	Q. prinoides Willd. × Q. stellata Wang.	N	6 ×	× Q. stelloides E. J. Palmer
12	Q. robur L. × Q. Ilex L.	N	11 × 12	× Q. Turneri Willd.
13	Q. Ilex L. × Q. sessiliflora	N	12 × 12	× Q. andleyensis Henry
14	Q. montana Willd. × Q. robur L.	N	12 × 11	× Q. Sargentii Rehd.
15	Q. macrocarpa Michx. × Q. undulata Torrey	N	12 (6) ×	× Q. Andrewsii Sarg.
16	Q. prinus L. × Q. macrocarpa Michx.	· N	× 6 (12)	× Q. byarsii Sudw.
17	Q. virginiana Miller × Q. macrocarpa Michx.	N	× 6 (12)	× Q. coloradensis Ashe
18	Q. lyrata Walter × Q. virginiana Miller	N, A	·	× Q. Comptonae Sarg.
19	Q. virginiana × Q. lyrata		·	
20	Q. alba L. × Q. Muehlenbergii Engel.	N	12 (6) × 6 (12)	× Q. Deamii Trel.
21	Q. alba L. & Q. stellata Wang.	N	12 (6) ×	× Q. Fernowii Trel.
22	Q. macrocarpa Michx. × Q. stellata Wang.	N	12 (6) ×	× Q. guadalupensis Sarg.
23	Q. stellata Margaretta (Ashe) Sarg. × Q. virginiana geminata (Small) Sarg.	N		× Q. Harbisonii Sarg.
24	Q. macrocarpa Michx. × Q. Muehlenbergii Engel.	N	6 (12) × 6 (12)	× Q. Hillii Trel.
25	Q. Douglasii Hook. & Arn. × Q. lobata Nee	N		× Q. jolonensis Sarg.
26	Q. durandii Buckl. × Q. stellata E. J. Palmer	N		× Q. Macnabiana Sudw.

Notes on hybrid	Country or region	Author and date of report or of origin	Other references	No.
6 seedlings.	U.S.A. (N. East)	Stout & Schreiner, (78) 1933		117
40 seedlings.	U.S.A. (N. East)	Stout & Schreiner, (78) 1933		118
82 seedlings, 2 very promising.	U.S.A. (N. East)	Stout & Schreiner, (78) 1933	80.	119
6 seedlings.	U.S.A. (N. East)	Stout & Sehreiner, (78) 1933		120
very rapid growth.	Europe, N. America	Lamb, 1916 (105)	9, 26.	121
fertile; intermediate; hybrid vigor variable; cultivated.	Germany	Klotzsch, 1854; (52) Geschwind, 1876	27, 28, 29, 44, 46, 66, 93, 94.	1
		(44)	28, 29, 46, 94.	2
fertile; several named varieties; vigorous growth; $F_1n=12$.	Europe, especially England	1765; Lamb, 1916 (105)	27, 28, 29, 32, 46, 52, 59, 66, 73, 74, 93, 94.	3
	Germany	Klotzsch, 1854 (52)	27, 28, 29, 44, 46, 93, 94.	4
	Spain	Natividade, 1937 (59)	28, 29, 31, 32.	5
	Spain	Natividade, 1937 (59)	28, 29, 31, 32.	6
hybrid vigor observed.	Russia	Kolesnikov, 1933 (48)	25, 27, 28, 29, 44, 46, 68, 93, 94.	7
also *Q. andleyensis* Henry; $F_1n=12$ (11).	Europe	Wetzel, 1929 (94)	27, 28, 29, 32, 46, 66, 93.	8
backcrossing probable; hybrid vigor not observed.	Mexico	Mueller, 1936 (106)		9
intermediate.	U.S.A. (S W.)	Palmer, 1937 (107)		10
intermediate in leaf characters.	U.S.A. (Mid W.)	Palmer, 1937 (107)	25. 29	11
var. *pseudoturneri* Henry.	England	b. 1785; Rehder, 1927 (66)	27, 29, 32, 74, 93.	12
parentage not certain.	Europe	Rehder, 1927 (66)	28, 29, 31, 32, 44.	13
differs from *Q. montana* chiefly by auriculate base of leaf and fewer lobes; very vigorous.	U.S.A.	b. 1830; Rehder, 1927 (66)	27, 29, 68, 93.	14
intermediate.	U.S.A., W. Okla.	Sargent, 1921 (67)	25, 29, 68, 74.	15
	U.S.A., S.W. Tenn.	Schreiner, 1937 (74)	25, 29, 68.	16
	U.S.A., E. Texas	Schreiner, 1937 (74)	25, 29, 68.	17
intermediate; cultivated; up to 100 ft. in height; see No. 19.	U.S.A., Ala., Miss., La., Texas	Sargent, 1921 (67)	74.	18
intermediate; partial fertility; hybrid vigor for fruit size; F_2 produced. See No. 18.	U.S.A.	1909; Yarnell, 1933 (102)		19
intermediate; introduced U.S.A. 1916.	U.S.A., Bluffton, Ind.	Sargent, 1921 (67)	25, 29, 66, 68, 74.	20
introduced U.S.A. b. 1898.	U.S.A., Ill., Md., Va., Miss.	Sargent, 1921 (67)	25, 29, 66, 68, 74.	21
intermediate.	U.S.A., S.E. Texas	Sargent, 1921 (67)	25, 29, 68, 74.	22
one tree.	U.S.A., Jacksonville, Fla.	Sargent, 1921 (67)	74.	23
	U.S.A., Ind., Mo.	Sargent, 1921 (67)	25, 29, 68, 74.	24
intermediate.	U.S.A., Jolon, Cal.	Sargent, 1921 (67)	74.	25
one tree.	U.S.A., Hampstead Co., Ark.	Schreiner, 1937 (74)		26

No.	Species involved	Nature of cross	Chromosome nos. (n) involved	Name of hybrid
	QUERCUS L. OAK (Continued)			
27	Q. arizonica Sarg. × Q. grisea Liebmann.	N		× Q. organensis Trel.
28	Q. dumosa Nutt. × Q. Engelmannii Greene	N		× Q. Macdonaldii Greene
29	Q. alba L. × Q. Prinus L.	N	12 (6) × 6	× Q. Beadlei Trel.
30	Q. alba L. × Q. macrocarpa Michx.	N	12 (6) × 12 (6)	× Q. Bebbiana Schn.
31	Q. alba × Q. macrocarpa	N	12 (6) × 12 (6)	× Q. Bebbiana Orpheusi
32	Q. alba L. × Q. prinoides	N	12 (6) × 6	× Q. Faxonii Trel.
33	Q. alba × Q. bicolor Willd.	N	12 (6) × 12	× Q. Jackiana Schn.
34	Q. alba L. × Q. montana L.	N	12 (6) × 12	× Q. Saulei Schn.
35	Q. bicolor Willd. × Q. macrocarpa Michx.		12 × 6 (12)	× Q. Schuettii Trel.
36	Q. Catesbaei Michx. × Q. cinerea Michx.	N		× Q. Ashei Trelease
37	Q rhombica Sarg. × Q. rubra L.	N		× Q. beaumontiana Sarg.
38	Q. cinerea Michx. × Q. laurifolia Michx.	N		× Q. atlantica Ashe
39	Q. Catesbaei Michx. × Q. rubra L.			× Q. blufftonensis Trelease
40	Q. marilandica Muench. × Q. velutina Lam.	N	6 × 6 (12)	× Q. Bushii Sarg.
41	Q. cinerea Michx. × Q. nigra L.	N	× 11 (12)	× Q. caduca Trelease
42	Q. marilandica Muench. × Q. cinerea Michx.	N	6 ×	× Q. caroliniensis Trelease
43	Q. rhombica Sarg. × Q. velutina Lam.	N	×16 (12)	× Q. Cocksii Sarg.
44	Q. imbricaria Michx. × Q. palustris Engel.	N	12 × 12 -	× Q. exacta Trelease
45	Q. ilicifolia Wang. × Q. Phellos L.	N		× Q. Giffordii Trel.
46	Q. marilandica Muench. × Q. texana Buckl.	N	6 ×	× Q. Hastingsii Sarg.
47	Q. borealis maxima (Marsh.) Ashe × Q. velutina Lam.	N	6 × 6 (12)	× Q. Hawkinsii Sudw.
48	Q. imbricaria Michx. × Q. velutina Lam.	N	× 6 (12)	× Q. Leana Nuttall
49	Q. rubra pagodaefolia (Elliott) Ashe × Q. Phellos L.	N		× Q. ludoviciana Sarg.
50	Q. Wislizenii A. DC. × Q. Kelloggii Newb.	N		× Q. morehus Kellogg
51	Q. Phellos L. × Q. rubra L.	N		× Q. subfalcata Trel.
52	Q. nigra L. × Q. Shumardii N. J. Palmer	N	11 (12) ×	× Q. neopalmeri Sudw.
53	Q. cinerea Michx. × Q. myrtifolia Willd.	N		× Q. oviedoensis Sarg.
54	Q. ellipsoidalis E. J. Hill × Q. velutina Lam.	N	× 6 (12)	× Q. paleolithicola Trel.
55	Q. borealis maxima (Marsh.) Ashe × Q. imbricaria	N	6 ×	× Q. runcinata (A. DC.) Engel.
56	Q. georgiana M. A. Curtis × Q. marilandica Muench.	N	× 6	× Q. Smallii Trel.
57	Q. marilandica Muench. × Q. nigra L.	N	6 × 11 (12)	× Q. sterilis Trel.
58	Q. cinerea Michx. × Q. rubra L.	N		× Q. subintegra Trel.
59	Q. rubra L. × Q. velutina Lam.	N	× 6 (12)	× Q. Sudworthii Trel.
60	Q. marilandica Muench. × Q. imbricaria Michx.	N	6 ×	× Q. tridentata Engel.
61	Q. arkansana caput-rivuli Ashe × Q. cinerea Michx.	N		× Q. venulosa Ashe

Notes on hybrid	Country or region	Author and date of report or of origin		Other references	No.
	U.S.A., N.M.	Schreiner, 1937	(74)		27
intermediate shrub.	U.S.A., Cal.	Sargent, 1921.	(67)		28
	U.S.A., Clarkton, N.C.	Sargent, 1921	(67)	25, 29, 68.	29
introduced U.S.A. b. 1880.	U.S.A., Vt., Ohio	Sargent, 1921	(67)	25, 29, 66, 68.	30
	U.S.A., Col.	Schantz, 1934	(72)	25, 29, 68.	31
intermediate.	U.S.A., Mass., Mich.	Sargent, 1921	(67)	25, 29, 68.	32
introduced U.S.A. 1916.	U.S.A., Boston, Mass.	Sargent, 1921	(67)	25, 29, 66, 68.	33
intermediate.	mainly N.E. U.S.A.	Sargent, 1921	(67)	25, 29, 66, 68.	34
leaves intermediate.	U.S.A., Wis.; Can., Que.	Sargent, 1921	(67)	25, 29, 66, 68.	35
	U.S.A., Georgia	Sargent, 1921	(67)	74.	36
Schreiner (74) states parentage as being *Q. obtusa* × *Q. rubra*.	U.S.A., Beaumont, Texas	Sargent, 1921	(67)	74.	37
	U.S.A., Carolina coast	Schreiner, 1937	(74)		38
	U.S.A., Bluffton, S.C.	Sargent, 1921	(67)	74.	39
	U.S.A., Southern	Sargent, 1921	(67)	25, 29, 68, 74.	40
	U.S.A., Southern	Sargent, 1921	(67)	25, 27, 28, 29, 46, 74, 93, 94.	41
	U.S.A., Ga., Texas, N.C.	Sargent, 1921	(67)	25, 29, 74.	42
	U.S.A., Pineville, La.	Sargent, 1921	(67)	25, 29, 68, 74.	43
cultivated 1889.	U.S.A., Mo., Ill., Ind., Pa.	Sargent, 1921	(67)	28, 29, 31, 32, 66, 68, 74.	44
	U.S.A., May's Landing, N.J.	Sargent, 1921	(67)	74.	45
	U.S.A., central Texas	Sargent, 1921	(67)	25, 29, 74.	46
Hawkin's Oak, single tree.	U.S.A., Huntingdon, Tenn.	Schreiner, 1937	(74)	25, 29, 68.	47
cultivated; introduced U.S.A. b. 1850; Lea Oak.	U.S.A., D.C., N.C., Mich., Ill., Mo.	Sargent, 1921	(67)	25, 29, 66, 68, 74.	48
var. *microcarpa* Rehd.; $F_1 n = 12$; single tree.	U.S.A., Peteville, La.	Sargent, 1921	(67)	29, 66, 68, 74.	49
	U.S.A., Cal.	Sargent, 1921	(67)	74.	50
var. *microcarpa* Sarg. of Dutch origin.	U.S.A., Ark., Texas, Ky., Ill., Miss.	Sargent, 1921	(67)	74.	51
single tree.	U.S.A., McNab, Ark.	Schreiner, 1937	(74)	27, 28, 29, 46, 93, 94.	52
	U.S.A., Oviedo, Fla.	Sargent, 1921	(67)	74.	53
	U.S.A., Mich., Ia., Ill.	Schreiner, 1937	(74)	25, 29, 68.	54
introduced U.S.A. 1883.	U.S.A., Mo., Ill.	Sargent, 1921	(67)	25, 29, 66, 74.	55
hybridity probable.	U.S.A., central Ga.	Sargent, 1921	(67)	25, 29, 74.	56
	U.S.A., Bladen Co., N.C.	Sargent, 1921	(67)	25, 27, 29, 28, 46, 74, 93, 94.	57
	U.S.A., coast Ga. to Fla.	Sargent, 1921	(67)	74.	58
also × *Q. Willdenoviana* Zabel.; single tree.	U.S.A., Covington, Tenn.	1880; Coffman	(74)	25, 29, 67, 68.	59
	U.S.A., Mo., Ill., Mich.	Sargent, 1921	(67)	25, 29, 74.	60
	U.S.A., Turkey Creek, Fla.	Schreiner, 1937	(74)		61

No.	Species involved	Nature of cross	Chromosome nos. (n) involved	Name of hybrid
	QUERCUS L. OAK (Continued)			
62	*Q. Catesbaei* Michx. × *Q. nigra* L.	N	× 11	× *Q. Walteriana* Ashe
63	*Q. borealis maxima* Ashe × *Q. velutina* Lam.	N	6 × 6 (12)	× *Q. Porterii* Trel.
64	*Q. coccinea* Muench. × *Q. ilicifolia* Wang.	N		× *Q. Robbinsii* Trel.
65	*Q. coccinea* Muench. × *Q. borealis maxima* Ashe	N	6 (4) × 6	× *Q. Benderi* Baenitz
66	*Q. ilicifolia* Wang. × *Q. marilandica* Muench.	N	× 6	× *Q. Brittonii* Davis
67	*Q. ilicifolia* Wang. × *Q. velutina* Lam.	N	× 6 (12)	× *Q. Rehderi* Trel.
68	*Q. marilandica* Muench. × *Q. Phellos* L.	N	6 ×	× *Q. Rudkinii* Brit.
69	*Q. cinerea* Michx. × *Q. laurifolia* Michx.	N		× *Q. sublaurifolia* Trel.
70	*Q. Phellos* L. × *Q. borealis maxima* Sarg.	N	× 6	× *Q. heterophylla* Michx.
71	*Q. Phellos* L. × *Q. palustris* L.	N	× 12	× *Q. Schochiana* Dieck
72	*Q. palustris* L. × *Q. borealis maxima* Sarg.	N	12 × 6	× *Q. Richteri* Boenitz
73	*Q. rubra pagodaefolia* Ashe × *Q velutina* Lam.	N		× *Q. Willdenoviana* Zabel
74	*Q. Catesbaei* Michx. × *Q. laurifolia* Michx.	⋅ N		× *Q. Mellichampii* Trel.
75	*Q. borealis maxima* Ashe × *Q. ilicifolia* Wang.	N	6 ×	× *Q. Lowellii* Sarg.
76	*Q. imbricata* Michx. × *Q. borealis maxima* Ashe	N	× 6	× *Q. runcinata* Engelm.
77	*Q. bicolor* Willd. × *Q. lyrata* Walt.		12 ×	× *Q. humidicola* E. J. Palmer
	ROBINIA L. LOCUST			
1	*R. pseudoacacia* L. × *R. neo-mexicana luxurians* Dieck	N	10 (11) × 10	× *R. Holdtii* Beiss.
2	*R. Kelseyi* Hutchins. × *R. pseudoacacia* L.	N	10 × 10 (11)	× *R. Slavinii* Rehd.
3	*R. pseudoacacia* L. × *R. hispida* L.	N	11 × 15	× *R. Margaretta* Ashe
4	*R. viscosa* Vent. × *R. pseudoacacia* L.	N	× 11	× *R. ambigua* Poir.
	SALIX L. WILLOW			
1	*S. viminalis* L. × *S. caprea* L.	A	19 × 19 (38)	× *S. Smithiana* Willd.
2	*S. viminalis* L. × *S. purpurea* L.	N, A	19 × 19	× *S. rubra* Huds.; Harrison willow.
3	*S. phylicifolia* × *S. viminalis*		44 × 19	
4	*S. caprea* L. × *S. lanata*		19 × 19	
5	*S. aurita* L. × *S. phylicifolia* L.		38 × 44	× *S. ludificans* B. White
6	*S. cinerea* L. × *S. phylicifolia* L.	N	38 × 44	× *S. Wardiana* B. White
7	*S. repens* L. × *S. viminalis* L.	N	19 × 19	× *S. Friesiana* Anders.
8	*S. caprea viminalis* × *S. americana* Hort.	A		
9	*S. viminalis* × *S. americana* Hort.	A	19 ×	
10	*S. purpurea* × *S. americana* Hort.	A	19 ×	
11	*S. alba* × *S. gracilis*	N	38 ×	× *S. coerulea*

Notes on hybrid	Country or region	Author and date of report or of origin	Other references	No.
	U.S.A., S.C., Ga., N.C., Fla., Ala.	Sargent, 1921 (67)	27, 28, 74, 93, 94.	62
	U.S.A., Mass., Pa., Ohio	Sargent, 1921 (67)	25, 29, 68,	63
introduced U.S.A. 1913.	U.S.A., North Easton, Mass.	Sargent, 1921 (67)	25, 27, 28, 29, 15, 66, 93, 94.	64
buds and leaves intermediate; originated in Europe, found in Silesia.	U.S.A., Boston, Mass.	b. 1900; Sargent, 1921 (67)	15, 25, 27, 29, 28, 66, 93, 94.	65
	U.S.A., N.Y., N.J.	Sargent, 1921 (67)	25, 29.	66
introduced U.S.A. 1905.	U.S.A., Mass.	Sargent, 1921 (67)	25, 29, 66, 68.	67
intermediate.	U.S.A., N.Y., N.J., N.C.	Sargent, 1921 (67)	25, 29.	68
	U.S.A., Ga., Miss.	Sargent, 1921 (67)		69
Q. velutina Lam. may be parent instead of *Q. borealis maxima* Sarg. Cultivated 1822.	U.S.A., N.J. to Tex.	Rehder, 1927 (66)	29, 25.	70
cultivated 1896.		Rehder, 1927 (66)	29, 68.	71
originated in Europe; occurs spontaneously with parents.	Europe, U.S.A.	b. 1900; Rehder, 1927 (66)	25, 29, 68.	72
parentage not certain.	Europe	Sargent, 1921 (67)		73
parentage not certain.	U.S.A., N.C., Fla.	Sargent, 1921 (67)		74
hybridity not certain.	U.S.A., Maine	Sargent, 1921 (67)	25, 29.	75
found with parents; introduced 1883.	Europe; U.S.A.	Rehder, 1927 (66)	25, 29.	76
intermediate.	U.S.A., Mo., Ill.	Palmer, 1937 (108)	29, 68.	77
chromosome pairing normal.	U.S.A., Col.	1890; Sargent, 1921 (67)	29, 66, 83, 95.	1
cultivated.	U.S.A., Col.	1915; Rehder, 1921 (66)	29, 83, 95.	2
(reduction division very irregular in *R. hispida* L.). Cultivated since 1920.	U.S.A., S.C.	Rehder, 1927 (66)	29, 50, 83.	3
also *R. dubia* Fouc., *R. intermedia* Soul.; cultivated.	U.S.A.	b. 1812; Rehder, 1927 (66)	29, 83.	4
F₂ produced; genetic study of leaves; cultivated since 1829.	Sweden	Heribert-Nilsson,1918 (52) 1931	9, 26, 27, 28, 35, 36, 41, 66, 90.	1
F₁ produced; long cultivation in Europe; immune to button gall.	Europe; U.S.A.	Wichura, 1865 (110)	7, 9, 26, 28, 35, 66.	2
	Russia	Bogdanov, 1935 (10)	9, 26.	3
	U.S.A.?	Blackburn & Harrison, (9) 1924	26, 28, 35.	4
cultivated since 1900; F₁n = 21–32.	N. Europe	Blackburn & Harrison, (9) 1924	26, 28, 35, 66.	5
cultivated since 1896; F₁n = 44.	Europe	Rehder, 1927 (66)	9, 26, 28, 35.	6
cultivated since 1829.	Europe	Rehder, 1927 (66)	9, 26, 28, 35.	7
stipules of *S. americana* dominant; higher in tannin content than *S. americana*.	Germany	Wettstein, 1931 (88)	90.	8
stipules of *S. americana* dominant; vigor of *S. americana*.	Germany	Wettstein, 1931 (88)	9, 26.	9
stipules of *S. americana* dominant.	Germany	Wettstein, 1931 (88)	9, 26, 90.	10
cricketbat willow.		Schreiner, 1937 (73)	9, 26, 37.	11

No.	Species involved	Nature of cross	Chromosome nos. (n) involved	Name of hybrid
	SALIX L. WILLOW (Continued)			
12	*S. nigra* Marsh. × *S. alba* L.	N	19 × 38	× *S. Hackensonii* Dode.
13	*S. pentandra* L. × *S. fragilis* L.	N	38 × 38	× *S. Meyeriana* Rostk.
14	*S. pentandra* L. × *S. alba* L.	N	38 × 38	× *S. Ehrhartiana* Sm.
15	*S. pentandra* L. × *S. purpurea* L.	N	38 × 19	× *S. heterandra* Dode.
16	*S. amygdalina* L. × *S. fragilis* L.	N	× 38	× *S. speciosa* Host.
17	*S. amygdalina* L. × *S. viminalis* L.	N	× 19	× *S. mollissima* Ehrh.
18	*S. fragilis* L. × *S. alba* L.	N	38 × 38	× *S. rubens* Schrank
19	*S. babylonica* L. × *S. alba* L.	N	× 38	× *S. sepulcralis* Simonk.
20	*S. babylonica* L. × *S. fragilis* L.	N	× 38	× *S. blanda* Anders.
21	*S. herbacea* L. × *S. formosa* Willd.	N		× *S. simulatrix* B. White.
22	*S. retusa* L. × *S. myrsinifolia* Salisb.	N		× *S. Cottetii* Lagger.
23	*S. caprea* L. × *S. myrsinifolia* Salisb.	N	19 (38) ×	× *S. latifolia* Forb.
24	*S. caprea* L. × *S. phylicifolia* L.	N	19 (38) × 44	× *S. laurina* Sm.
25	*S. aurita* L. × *S. cinerea* L.	N	38 × 38	× *S. multinervis* Doell.
26	*S. aurita* L. × *S. repens* L.	N	38 ×	× *S. ambigua* Ehrh.
27	*S. myrsinifolia* Salisb. × *S. hastata* L.	N		× *S. Mielichhoferi* Saut.
28	*S. phylicifolia* L. × *S. myrsinifolia* Salisb.	N	44 × ·	× *S. tetrapla* Walker
29	*S. cordata* Muhlenb. × *S. sericea* Marsh.	N		× *S. myricoides* Muhlenb.
30	*S. daphnoides* Vill. × *S. caprea* L.	N	× 19 (38)	× *S. Erdingeri* Kern.
31	*S. lapponum* L. × *S. caprea* L.	N	× 19 (38)	× *S. Laestadiana* Hartm.
32	*S. viminalis* L. × *S. aurita* L.	N	38 × 38	× *S. fruticosa* Doell.
33	*S. viminalis* L. × *S. cinerea* L.	N	38 × 38	× *S. holosericea* Willd.
34	*S. incana* Schrank × *S. caprea* L.	N	× 19 (38)	× *S. Seringeana* Gaud.
35	*S. incana* Schrank × *S. aurita* L.	N	× 38	× *S. patula* Ser.
36	*S. incana* Schrank × *S. repens* L.	N	× 19	× *S. subalpina* Forb.
37	*S. incana* Schrank × *S. daphnoides*	N		× *S. Reuteri* Moritzi.
38	*S. purpurea* L. × *S. caprea* L.	N	19 × 19 (38)	× *S. Wimmeriana* Gren. & Godr.
39	*S. purpurea* L. × *S. grandifolia*	N	19 ×	× *S. austriaca* Host.
40	*S. purpurea* L. × (*S. aurita* L. × *S. phylicifolia* L.)	N	19 × (21-32)	× *S. sequitertia* B. White
41	*S. purpurea* L. × *S. cinerea* L.	N	19 × 38	× *S. Pontederana* Willd.
42	*S. purpurea* L. × *S. repens* L.	N	19 × 19	× *S. Doniana* Sm.
	TAXUS L. YEW			
1	*T. cuspidata* Sieb. & Zucc. × *T. baccata* L.	N	12 ×	× *T. media* Rehd.
2	*T. cuspidata* Sieb. & Zucc. × *T. canadensis* Marsh.	N	12 ×	× *T. Hunnewelliana* Rehd.
	TILIA L. BASSWOOD, LINDEN			
1	*T. cordata* Mill. × *T. platyphyllos* Scop.	N	36 × 40	× *T. vulgaris* Hayne
2	*T. americana* × *T. argentea*		× 40	
3	*T. platyphyllos* Scop. × *T. glabra* Vent.		40 ×	× *T. carlsruhensis* Simonk.
4	*T. cordata* Mill. × *T. dasystyla* Stev.	N	36 ×	× *T. euchlora* K. Koch.
5	*T. cordata* Mill. × *T. glabra* Vent.	N	36 ×	× *T. flavescens* A. Br.

Notes on hybrid	Country or region	Author and date of report or of origin		Other references	No.
		b. 1890; Rehder, 1927	(66)	9, 26.	12
cultivated in Europe, 1829.	Europe	Rehder, 1927	(66)	9, 26.	13
also *S. hexandra* (various authors);	Europe	Rehder, 1927	(66)	9, 26.	14
cultivated Europe, 1894.					
parentage not certain; cultivated	Caucasus	Rehder, 1927	(66)	9, 26.	15
in Caucasus since 1910.					
cultivated in Europe since 1821.	Europe	Rehder, 1927	(66)	9, 26.	16
cultivated in Europe, 1809.	Europe	Rehder, 1927	(66)	9, 26.	17
intermediate; very variable.		Rehder, 1927	(66)	9, 26.	18
more vigorous, less weeping than		b. 1864; Rehder, 1927	(66)	9, 26.	19
S. bab.					
weeping habit like *S. babylonica*.		b. 1830; Rehder, 1927	(66)	9, 26.	20
S. polaris Wahl. similar;	Switzerland	Rehder, 1927	(66)		21
cultivated since 1922.					
S. serpyllifolia Scop. a related	Europe (Alps)	Rehder, 1927	(66)		22
var.; cultivated since 1905.					
Harrison (36) found tetraploid	Europe	Rehder, 1927	(66)	9, 26, 27, 36.	23
caprea; cultivated since 1829.					
Harrison (36) found tetraploid		Rehder, 1927	(66)	9, 26, 27, 36.	24
caprea; cultivated since 1809.					
cultivated since 1873.	Europe	Rehder, 1927	(66)	9, 26	25
cultivated 1872.	Europe	Rehder, 1927	(66)	9, 26.	26
cultivated 1888.	Europe	Rehder, 1927	(66)		27
cultivated 1829.	Europe	Rehder, 1927	(66)	9, 26.	28
cultivated 1880.	U.S.A., Mass. to	Rehder, 1927	(66)		29
	Wis., Kan.				
cultivated 1872.	Europe	Rehder, 1927	(66)	9, 26, 27, 36.	30
cultivated 1873.	Europe	Rehder, 1927	(66)	9, 26, 27, 36.	31
cultivated 1829.	Europe	Rehder, 1927	(66)	9, 26.	32
cultivated 1829.	Europe	Rehder, 1927	(66)	9, 26.	33
cultivated 1872.	Europe	Rehder, 1927	(66)	9, 26, 27, 36.	34
cultivated 1829.	Europe	Rehder, 1927	(66)	9, 26.	35
cultivated 1829.	Europe (Alps)	Rehder, 1927	(66)	9, 26.	36
cultivated 1870.	Europe	Rehder, 1927	(66)		37
cultivated 1872.	Europe	Rehder, 1927	(66)	9, 26, 27, 36.	38
cultivated 1870.	Europe	Rehder, 1927	(66)	9, 26.	39
cultivated 1900.	Europe	Rehder, 1927	(66)	9, 26.	40
cultivated 1829.	Europe	Rehder, 1927	(66)	9, 26.	41
cultivated 1829.	Europe	Rehder, 1927	(66)	9, 26.	42
intermediate; leaves similar to	U.S.A.	1900; Rehder, 1927	(66)	71.	1
T. cuspidata but more distinctly					
2-ranked.					
resembles *T. cuspidata*, but of	U.S.A.	1900; Rehder, 1927	(66)	71.	2
more slender habit and with					
narrower leaves.					
fertile; occur mainly as planted	U.S.A., Europe	Larsen, 1937	(52)	27, 29, 66, 86.	1
trees.					
silver leaf of *argentea*, vigor of *amer.*	Russia	Kolesnikov, 1933	(48)	29, 86.	2
		Rehder, 1927	(66)	27, 29, 81, 86.	3
parentage doubtful.		Rehder, 1927	(66)	29, 86.	4
also × *T. Spaethii* Spaeth, and ×		Rehder, 1927	(66)	29, 86.	5
T. floribunda A. Br.					

No.	Species involved	Nature of cross	Chromosome nos. (n) involved	Name of hybrid
	TILIA L. BASSWOOD, LINDEN (Continued)			
6	T. glabra Vent. × T. petiolaris Hook.	N		× T. Moltkei Spaeth
7	T. petiolaris Hook. × T. euchlora K. Koch.	N		× T. orbicularis Jouin
8	T. Miyabei Jack. × T. japonica Simonk.	N		× T. nosiricola Hisauti
	TSUGA CARR. HEMLOCK			
1	T. Mertensiana Sarg. × T. heterophylla Sarg.	N		× T. Jeffreyi Henry
	ULMUS L. ELM			
1	U. glabra Huds. × U. foliacea Gilib.	N		× U. hollandica Mill.
2	U. campestris × U. effusa	A	14 (+) ×	
3	U. americana × U. laevis	A	28 (14) × 14	
4	U. montana × U. nitens		14 ×	× U. major Smith
5	U. glabra × U. montana	N	× 14	Huntingdon elm
6	U. glabra Huds. × U. pumila L.	N	× 15	× U. arbuscula Wolf

Discussion

A detailed discussion of the data summarized in the above list will not be given, since it would require much space, and would probably be, at best, somewhat unwieldly and loosely connected. Furthermore, it is felt that the data as summarized hardly require such a discussion. Only a few general points, therefore, will be touched upon.

The above list includes many presumed hybrids which have been described by taxonomists on a purely morphological basis without definite experimental evidence as to parentage. Such presumed hybrids must be regarded with some caution, especially in genera in which experimental hybridization has not been done. On the other hand, it should be recognized that the description of these hybrids indicates that in the genera in question there must exist a considerable degree of integradation between species, which, in turn, indicates the probability that hybridization has occurred.

The author is aware that the value of including such hybrids is a debatable point. However, after some consideration it was decided to include hybrids of this class in instances where the assumed parentage appeared to be accepted generally as being reasonably certain. This action seemed best to serve one of the main objectives of the work—to indicate the species in a given genus, between which crossing is most likely to occur naturally or to be effected artificially.

Notes on hybrid	Country or region	Author and date of report or of origin	Other references	No.
cultivated since 1830.	Can. to N.C., west to Minn., Mo., U.S.A.	b. 1800; Rehder, 1927 (66)		6
	U.S.A.	1870; Rehder, 1927 (66)		7
	Japan	Hisauti, 1937 (42)		8
also *T. Mertensiana Jeffreyi* Schneid.; introduced U.S.A. 1851.	U.S.A.	Rehder, 1927 (66)		1
fertile; many named forms; tendency to be intermediate; vigorous growth; Fm=14.	Europe, especially England; U.S.A.	Rehder, 1927 (66)	52, 54, 69.	1
more vigorous than parents.	Germany	Klotzsch, 1854 (52)	29, 49, 54, 85.	2
	U.S.A.	Sax, 1933 (69)	29, 49, 85.	3
× *U. serpentina* Henry, a pendulous var. of *U. major*.	England	Schreiner, 1937 (74)	29, 49, 54, 85.	4
very rapid growth (disc. by Rehder under No. 1).		Schreiner, 1937 (73)	29, 37, 49, 54, 85.	5
shrub habit of *U. pumila* dominant; cultivated.	U.S.A.	1902; Rehder, 1927 (66)	85.	6

A number of named hybrids were not included because evidence as to parentage appeared to be lacking or of a very doubtful nature. These are as follows:

  × *Acer hybridum* Spach. (74)

  × *ramosum* Schwer. (*A. pseudoplatanus* L. × ?) (66)

  × *rotundilobum* Schwerin (74)

  × *sericeum* Schwer. (*A. pseudoplatanus* L. × ?) (66)

  × *Crategus celsiana* Bosc. (*C. pentagyna* Waldst. & Kit. × ?) (66)

  × *grignonensis* Mouillef. (*C. pubescens* Steud. × ?) (66)

  × *persistens* Sarg. (possibly hybrid of *C. crus-galli* L.) (66)

  × *sorbifolia* Lge. (*C. oxyacantha* L. × ?) (66)

  × *Fraxinus elonza* Dippel. (74)

  × *Larix Marschlinsii* (74)

  × *Quercus demarei* Ashe (74)

  × *dubia* Ashe (74)

  × *Koehnii* (28, 94)

  × *mallichampii* Trelease (67, 74)

  × *podophylla* Trel. (74)

  × *Salix renecia* Dode. (*S. cinerea* L. × ?) (66)

Attention is drawn to the fact that in *Quercus* there appears to be little or no crossing between the two sections of the genus, white oaks and black oaks

(Sargent's classification (67)).   In the list, crosses 1 to 35, inclusive, and cross 77 involve only white oaks, while crosses 36 to 76, inclusive, involve only black oaks.

A similar condition exists in *Pinus*.   Here all crosses except No. 13 involve hard (or pitch) pines exclusively.   No. 13 involves two soft pines.

These points are important to the breeder, since they indicate possible limitations in intercrossability among certain groups of species in the genera in question.   They also tend to give biological support to the taxonomists' division of these genera into sections.

On the other hand, in certain complex genera, *e.g.*, *Populus*, there appears to be little limitation to the crossing of species belonging to different sections— this information is, of course, very important to the breeder.

It is hoped that it will be possible within a few years to revise and extend the present work.   To that end, the author would be greatly obliged to learn of omissions and inaccuracies in the present work, and to receive reprints of future publications on forest-tree hybridization for use in the proposed work.

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EFFECTS OF PLANT AND ANIMAL HORMONES ON SEEDS DAMAGED BY FORMALDEHYDE[1]

By N. H. Grace[2]

Abstract

Marquis wheat was immersed in solutions of formaldehyde and formaldehyde containing either naphthylacetic acid or oestriol in concentrations of 0.1, 1, 5, and 10 p.p.m., and germinated on blotting paper or grown in soil at a temperature of from 70 to 75° F. A measure of physiological activity was shown by both chemicals from the results of germination on blotting paper; the activity of the two chemicals was of the same order. Growth in soil failed to show anything but injurious effects from formaldehyde.

In another experiment, Marquis wheat was sprinkled with solutions of formaldehyde and indolylacetic acid and grown in soil at a temperature of from 50 to 55° F. The sprinkling treatment, which supplied one part of indolylacetic acid to a million parts of wheat by weight, reduced formaldehyde injury in a statistically significant manner. Lower concentrations of the chemical did not reduce injury to a significant extent.

A recent publication described the results of experiments in which plant cuttings were treated with dusts and solutions containing oestrone and indolylbutyric acid (3). In view of the results from the treatment of plant cuttings, it was of interest to consider other methods of determining the physiological activity of oestrogenic substances. It has been shown that a number of chemicals reduce seed injury arising from disinfection with formaldehyde solutions and other similar treatments (2). In consequence, this method was chosen in an effort to determine the activity of an oestrogenic hormone, oestriol, and incidentally, to investigate further the effects of indolylacetic and naphthylacetic acids on formaldehyde injury.

Experimental

Solutions were prepared by dissolving 0.05 gm. of each of the chemicals in 1 cc. of 95% alcohol and diluting with formaldehyde solution to give 50 p.p.m. (parts per million) in 1 : 320 formaldehyde. Subsequent dilution with formaldehyde of the same concentration permitted ready preparation of the various concentrations required.

In the first experiment, 50-gm. samples of Marquis wheat were immersed in 50 cc. of a 1 : 320 solution of formaldehyde* (37% by weight of the gas) for 5 min. (2). The samples were drained for 2 min., placed on filter paper and covered with inverted cans for 4 hr. Each sample was then loosely wrapped in small pieces of canvas to prevent aeration and planted approximately 24 hr. after treatment.

The series of treatments included untreated and formaldehyde-treated controls and concentrations of 0.1, 1, 5, and 10 p.p.m. of each of the chemicals separately in formaldehyde, making 10 treatments in all. Eight replicates of 50 seeds were planted on blotting paper in a germinator maintained at a temperature of approximately 65° F. Ten replicates of 50 seeds were planted in small cardboard flats and kept in a greenhouse at a temperature of approximately 72° F. Germination counts were made five and nine days after planting on blotting paper; the number of seminal roots was counted and length of each stem measured from the seed to the tip of the longest leaf, and expressed as number of roots and length of stem per seedling. Germination rates computed by Bartlett's method (1), final germination, and air-dry weights of tops and roots for the plants from 50 seeds, were determined from the plants grown in soil. The plants were washed out of soil 23 days after planting, placed in an oven at 95° for two hours, and conditioned for one week in the laboratory.

The second experiment involved the use of five treatments comprising untreated and formaldehyde controls and 0.1, 1, and 5 p.p.m. of indolylacetic acid in formaldehyde solution. Twenty-five-gram samples of Marquis wheat were sprinkled with 5 cc. of the 1 : 320 commercial formaldehyde (37% by weight of the gas) solutions. Sprinkling with solutions of the concentrations mentioned gave seed treatments with 0.02, 0.2, and 1 parts of indolylacetic acid per million parts of seed by weight. The samples of treated seed were covered with inverted cans for four hours and then wrapped in canvas overnight. Ten replicates of 50 seeds of each of the five treatments were planted in soil in small cardboard flats, approximately 24 hr. after treatment, and held in a greenhouse at a temperature between 50 and 55° F. The experiment was arranged in the form of two contiguously placed Latin squares each containing five replicates of the five treatments. Germination rates, final counts, and air-dry top and root weights 36 days after planting, were determined in the manner already described.

TABLE I

ANALYSIS OF VARIANCE OF RESPONSE OF MARQUIS WHEAT TREATED WITH SOLUTIONS OF OESTRIOL AND NAPHTHYLACETIC ACID IN FORMALDEHYDE AND GERMINATED ON BLOTTING PAPER

Source of variance	Degrees of freedom	Mean square			
		Germination at		Per seedling	
		Five days	Nine days	Number of seminal roots	Stem length
Replicates	7	304.5***	65.3***	0.09997	377.67***
Treatments	9	150.5***	26.8**	0.62970***	381.82***
Error	63	18.5	8.7	0.08181	73.89 ·

** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

TABLE II

EFFECTS OF FORMALDEHYDE–HORMONE TREATMENTS ON THE RESPONSES OF MARQUIS WHEAT GERMINATED ON BLOTTING PAPER

	Controls		Formaldehyde and naphthylacetic acid, p.p.m.				Mean of all treatments with naphthylacetic acid	Formaldehyde and oestriol, p.p.m.				Mean of all treatments with oestriol	Necessary differences, 5% level
	Untreated	Formaldehyde	0.1	1	5	10		0.1	1	5	10		
Germination at 5 days[4]	38.1	21.6	30.0	27.4	33.2	30.9	30.4	28.8	30.0	31.0	26.4	29.0	4.3[1] 3.4[2] 2.1[3]
Germination at 9 days[4]	43.0	41.3	46.4	43.5	46.9	45.6	45.5	44.8	45.9	46.8	44.4	45.5	2.9[1] 2.3[2] 1.5[3]
Number of seminal roots per seedling	3.50	4.18	4.41	4.08	4.31	4.42	4.31	4.39	4.43	4.18	4.24	4.31	0.29[1] 0.23[2] 0.14[3]
Length of stem per seedling, mm.	100.5	76.9	79.1	80.0	84.5	85.3	82.2	87.5	83.6	81.8	77.0	82.5	8.8[1] 6.8[2] 4.3[3]

[1] Necessary difference between individual treatments.
[2] Necessary difference between formaldehyde control and means.
[3] Necessary difference between means of treatments with naphthylacetic acid and oestriol.
[4] Number of seeds germinated of 50 planted.

Results

FORMALDEHYDE–HORMONE TREATMENT OF MARQUIS WHEAT

Results from Growth in the Germinator on Blotting Paper

In Table I are given results for the analyses of variance, and in Table II, treatment means for the data on germination at five and nine days, and the number of seminal roots and stem lengths per seedling for wheat germinated on blotting paper. Treatment effects were significant for each of the four sets of observations. Germination counts at five days demonstrated significant injury from formaldehyde treatment and reduction of injury with each of the treatments with plant and animal hormones, the difference between the means for all naphthylacetic acid and oestriol treatments was not significant. While the final nine-day germination count failed to show significant reduction on treatment with formaldehyde alone, all the treatments gave greater germination than was shown by the formaldehyde control. Germination means for each of the four treatments are greater than the value for formaldehyde, but no significant difference is shown between naphthylacetic acid and oestriol. None of the hormone treatments differed from the formaldehyde treatment, which effected a significant increase in the number of seminal roots per seedling. Furthermore, a marked reduction in stem length was caused by formaldehyde treatment. Treatment with 0.1 p.p.m. oestriol in formaldehyde significantly increased stem length; none of the other treatments differed from formaldehyde alone.

Results from Growth in Soil

The analyses of variance and treatment means for germination rates, final germination count, and air-dry top and root weights are given in Tables III and IV. Treatment effects were highly significant for each of the four characters considered; however, significance was related to the depressing effects of formaldehyde treatment. The effect of 10 p.p.m. oestriol in formaldehyde on both top and root weights approached a significant reduction in injury.

TABLE III

ANALYSIS OF VARIANCE OF RESPONSE OF MARQUIS WHEAT TREATED WITH SOLUTIONS OF OESTRIOL AND NAPHTHYLACETIC ACID IN FORMALDEHYDE AND GROWN IN SOIL

Source of variance	Degrees of freedom	Mean square			
		Germination		Air-dry weight	
		Rates	Final count	Tops	Roots
Replicates	9	0.05134***	130.58***	0.03506**	0.00378**
Treatments	9	0.04804***	194.12***	0.08194***	0.01281***
Error	81	0.00268	28.03	0.01000	0.00106

** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

TABLE IV

EFFECTS OF FORMALDEHYDE–HORMONE TREATMENTS ON THE RESPONSES OF MARQUIS WHEAT GROWN IN SOIL

	Controls		Formaldehyde and naphthylacetic acid, p.p.m.				Mean of all treatments with naphthylacetic acid	Formaldehyde and oestriol, p.p.m.				Mean of all treatments with oestriol	Necessary differences, 5% level
	Untreated	Formaldehyde	0.1	1	5	10		0.1	1	5	10		
Germination rates	0.857	0.657	0.662	0.648	0.648	0.637	0.649	0.633	0.617	0.625	0.672	0.637	0.046[1] 0.036[2] 0.023[3]
Final germination from 50 seeds	46.7	32.5	34.4	31.6	33.3	31.4	32.7	35.1	34.2	33.4	35.9	34.7	4.7[1] 3.7[2] 2.4[3]
Air-dry weight of tops from 50 seeds, gm.	0.909	0.610	0.654	0.582	0.629	0.628	0.623	0.660	0.645	0.658	0.698	0.665	0.089[1] 0.070[2] 0.044[3]
Air-dry weight of roots from 50 seeds, gm.	0.253	0.137	0.157	0.140	0.145	0.140	0.146	0.148	0.147	0.126	0.165	0.147	0.029[1] 0.023[2] 0.014[3]

[1] *Necessary difference between individual treatments.*

[2] *Necessary difference between formaldehyde control and means.*

[3] *Necessary difference between means of treatments with naphthylacetic acid and oestriol.*

While oestriol treatment gave a greater weight of tops than naphthylacetic acid treatment, the difference is just insufficient for significance. Apart from these suggestions of possible effects from hormone treatment, the only clearly demonstrated effect was injury from treatment with formaldehyde alone.

FORMALDEHYDE–INDOLYLACETIC ACID TREATMENT OF MARQUIS WHEAT

The results secured from analysis of variance and the effects of treatments on germination rates, final germination counts, and air-dry weights of tops and roots, for plants grown in soil, are given in Tables V and VI. Formaldehyde treatment effected significant reduction in each case. Treatment with 5 p.p.m. indolylacetic acid solution (or 1 p.p.m. of the weight of the seed)

TABLE V

ANALYSIS OF VARIANCE OF RESPONSE OF MARQUIS WHEAT TREATED WITH SOLUTIONS OF FORMALDEHYDE AND INDOLYLACETIC ACID

		Mean square			
Source of variance	Degrees of freedom	Final germination count	Germination rate $(\times 10^4)$	Air-dry weight	
				Tops $(\times 10^3)$	Roots $(\times 10^3)$
Squares	1	32.00*	0.72	203.64***	140.45***
Rows	8	2.34	28.91	18.58*	9.09
Columns	8	4.64	21.86	12.56	8.80
Treatments	4	31.33**	512.80***	69.17***	59.69***
Treatments × squares	4	7.65	22.45	3.29	5.84
Error	24	5.72	13.42	7.59	6.02

* Exceeds mean square error, 5% level of significance.
** Exceeds mean square error, 1% level of significance.
*** Exceeds mean square error, 0.1% level of significance.

TABLE VI

EFFECTS OF FORMALDEHYDE–INDOLYLACETIC ACID TREATMENTS ON THE RESPONSES OF MARQUIS WHEAT GROWN IN SOIL

	Controls		Concentration of indolylacetic acid in 1 : 320 formaldehyde, p.p.m.			Necessary difference, 5% level
	Untreated	Formal-dehyde	0.1	1	5	
Germination[1]	46.2	41.5	42.8	42.5	43.2	2.2
Germination rates	0.767	0.592	0.611	0.606	0.627	0.034
Air-dry weight of plants from 50 seeds, gm.						
Tops	1.20	0.98	1.04	1.04	1.09	0.08
Roots	0.63	0.46	0.48	0.44	0.49	0.07

[1] Number of seeds germinated of 50 planted.

significantly increased the germination rate and the air-dry weight of tops from 50 seeds, as compared with the formaldehyde control, but did not increase root weights or the final germination to a significant extent. The lower indolylacetic acid concentrations failed to give any significant effects.

Discussion

Marquis wheat treated with solutions of formaldehyde and formaldehyde naphthylacetic acid or oestriol, and germinated on blotting paper, indicated a measure of physiological activity for both chemicals. However, there were no significant differences between the means for all concentrations of each chemical, and the results indicated that oestriol reduced formaldehyde injury to about the same extent as naphthylacetic acid. The results from growth in soil failed to show any reduction in formaldehyde injury from either naphthylacetic acid or oestriol.

The results of germination (9-day counts) on blotting paper indicated that even though formaldehyde treatment failed to reduce germination significantly, highly significant effects were shown by the decrease in length of the stem and increase in the number of seminal roots per seedling. Effects of formaldehyde injury were more marked on plants grown in soil.

In the second experiment, when Marquis wheat was sprinkled, a treatment of 1 p.p.m. of indolylacetic acid reduced injury in a statistically significant manner, but 0.02 or 0.2 p.p.m. of the hormone was inadequate. This result is in general agreement with earlier work on plants grown in soil (2). The positive effects from formaldehyde–indolylacetic acid treatment on plants grown in soil may have been due to differences in effectiveness of this chemical and naphthylacetic acid, or to the different temperatures under which growth occurred.

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EFFECT OF VARIOUS CONDITIONS OF STORAGE ON BAKING QUALITY OF FLOUR[1]

By A. G. McCalla[2], J. D. McCaig[3], and A. D. Paul[4]

Abstract

Flour stored in sealers in a refrigerator at 2° C. did not change in quality for 22 months after milling. Similar flours stored in sealers at room temperature deteriorated significantly within three months, while a third lot stored in small bags at room temperature showed first a definite improvement and then rapid deterioration. At the end of 22 months flours stored in sacks were much the poorest. Acidity of all flours increased with storage, but this determination was of little value in estimating flour quality.

Introduction

Routine tests carried out at this institution showed that flours varied in keeping properties during storage, depending on the storage conditions and on the variety and source of wheat from which they were milled (1, 7). Flours stored for 12 months in air-tight sealers maintained their quality better than did flours stored in small sacks, despite the fact that the latter contained much less moisture than the former. In general, flours milled from wheats grown on the black loam at Edmonton maintained their quality better than flours milled from the same varieties of wheat grown on the grey podsolic loam typical of much of western and northern Alberta.

Studies on flour storage in general show that under ideal conditions of packaging, temperature, humidity, etc., high grade flour will maintain its quality for many months, and even years (3, 5–8). Under less favourable conditions, however, deterioration may be marked in a few months.

The most comprehensive recent study has been carried out by Fisher *et al.* (5). They found a periodicity in improvement and deterioration of quality, and in various chemical properties. In general, however, the results with respect to keeping properties as affected by storage conditions and nature of flours confirm those obtained in earlier work. No definite "best" conditions of storage can be determined as a result of past experience under experimental conditions.

The work here reported was carried out with the object of checking previous results, and of following more closely the changes in flour quality with storage.

Material and Methods

Four varieties of wheat; Garnet, Marquis, Red Bobs, and Reward, each grown in 1936 at Edmonton and Fallis (50 miles west of Edmonton) were milled on an experimental mill to produce long patent flours. The data pertinent to this study are given in Table I. All samples were sound and well matured, those from Fallis being unusually high in protein because the growing season was very dry. In average seasons, the protein content of these varieties of wheat would be 2 to 3% lower than in 1936.

TABLE I

DATA PERTAINING TO ORIGINAL QUALITY OF WHEATS USED IN STUDY

Station	Type of soil	Variety	Weight, per bushel, lb.	Commercial grade	Protein, %	Loaf volume, cc.
Edmonton	Black loam	Garnet	65.0	1 C.W. Gar.	14.1	325
		Marquis	65.5	2 Nor.	15.0	373
		Red Bobs	64.0	1 Nor.	14.3	378
		Reward	65.0	1 Nor.	16.5	448
Fallis	Podsolic loam	Garnet	65.5	1 C.W. Gar.	12.5	263
		Marquis	65.0	3 Nor.	12.3	283
		Red Bobs	65.0	2 Nor.	12.0	300
		Reward	67.0	2 Nor.	14.7	393

The flour was stored in large cans for one month after milling. This is the regular practice in this laboratory, chiefly because the experimental milling and baking are done by the same man. It has also been found that most flours stored for one month before baking produce decidedly better bread than if baked immediately after milling. Baking tests were then made, and each sample of flour was divided into three lots. The conditions of storage were as follows:

Lot 1: Stored in glass sealers which were kept in a refrigerator at 2° C. The sealers were removed only to permit sampling.

Lot 2: Stored in sealers on open shelves in the laboratory. Temperature varied between 16 and 28° C. at different times of the year.

Lot 3: Stored in small cotton sacks in the same room as Lot 2.

The flour contained approximately 13.5% moisture at the beginning of the experiment. At the end of 22 months the moisture content of the three lots was approximately 13.5, 11.0, and 8.0%.

All baking tests were carried out using 50 grams of flour and the malt-phosphate-bromate formula (2).

Acidity was determined by the Greek method (4), using tincture of curcuma as indicator. Results are reported as the volume of $N/50$ sodium hydroxide required to neutralize 10 cc. of the alcoholic extract.

TABLE II

LOAF VOLUME OF BREAD BAKED FROM FLOUR STORED UNDER VARIOUS CONDITIONS

Station	Variety	Original volume, July 1937	Condition of storage								
			Sealers at 2° C.			Sealers at room temperature			Sacks at room temperature		
			December 1937	July 1938	April 1939	December 1937	July 1938	April 1939	December 1937	July 1938	April 1939
Edmonton	Garnet	325	300	258	284	260	220	250	379	270	162
	Marquis	373	358	393	337	353	344	334	445	279	217
	Red Bobs	378	418	426	420	369	373	362	473	344	240
	Reward	448	465	386	430	408	376	365	485	342	251
	Mean	381	385	366	368	348	328	328	446	309	218
Fallis	Garnet	263	280	253	302	260	215	237	300	210	140
	Marquis	283	320	300	275	290	273	275	344	243	172
	Red Bobs	300	337	315	320	300	288	293	351	290	175
	Reward	393	408	437	392	367	310	327	457	313	165
	Mean	310	336	326	322	304	272	283	363	264	163

Results

Baking tests were carried out 1, 4, 6, 9, 13, 18, and 22 months after the flours were milled. Results for loaf volume of individual samples at 1, 6, 13, and 22 months are given in Table II, and the results of analyses of variance for all data in Table III.

TABLE III

ANALYSIS OF VARIANCE, LOAF VOLUME OF BREAD STORED UNDER THREE SETS
OF CONDITIONS

Variance due to	D.f..	Mean squares		
		Sealers at 2° C.	Sealers at room temperature	Sacks at room temperature
Station	1	46,980*	38,587*	72,216**
Variety	3	44,963*	35,233*	24,343**
Time of storage	6	622	2,652**	50,486**
Station × Variety	3	3,332	1,775**	671
Station × Time	6	366	411	1,316*
Variety × Time	18	405	338·	579
Station × Variety × Time	18	2,823	184	387
Total	55			

* Significant beyond the 5% point.
** Significant beyond the 1% point.

The effects of station and variety were significant for all conditions of storage. These effects were largely the result of differences in protein content of the wheat from which the flour was milled, although the Garnet samples fell somewhat below the general protein-loaf-volume level. Contrary to expectations from the results of earlier studies (1, 7), there was no significant interaction between variety and station, except in the case of flour stored in sealers. Even with this flour the magnitude of the interaction was small, though significant, and attributable chiefly to the behaviour of one variety, Red Bobs.

The effect of time of storage was very different with the three conditions of storage.

Since there were virtually no significant interactions, one graph drawn from the means for the four varieties and two stations presents the important results obtained in the study. This is given in Fig. 1. The slight increase in mean loaf volume for the flours stored in the refrigerator is not significant, but the decrease in volume for the flours stored in sealers on open shelves is. The flour stored in sacks shows what has been considered as a normal course in aging, that is, first an improvement, and then a sharp decrease, in quality (3).

The results of earlier studies showed that acidity of all flours increased with age, but that the increases were not parallel to changes in quality. Acidity determinations were made 1, 6, 13, and 22 months after milling. The results are presented in Table IV and Fig. 2. The results of analyses of variance

TABLE IV

ACIDITIES OF FLOUR (CC. OF N/50 SODIUM HYDROXIDE) STORED UNDER VARIOUS CONDITIONS

Station	Variety	Original, July 1937	Condition of storage								
			Sealers at 2° C.			Sealers at room temperature			Sacks at room temperature		
			December 1937	July 1938	April 1939	December 1937	July 1938	April 1939	December 1937	July 1938	April 1939
Edmonton	Garnet	1.25	1.71	1.68	1.96	2.00	2.07	2.50	1.57	2.07	2.43
	Marquis	1.07	1.46	1.54	1.57	1.43	2.11	1.79	1.39	1.50	1.71
	Red Bobs	1.11	1.61	1.79	1.50	2.07	1.79	1.79	1.46	1.86	2.03
	Reward	1.18	1.43	1.68	1.57	1.57	1.86	1.96	1.50	1.79	2.06
	Mean	1.15	1.55	1.67	1.65	1.77	1.96	2.01	1.65	1.81	2.06
Fallis	Garnet	1.14	1.61	1.61	1.50	1.64	1.79	2.07	1.68	1.86	1.86
	Marquis	1.36	1.64	1.86	2.00	2.14	2.25	2.64	1.86	2.14	2.21
	Red Bobs	1.36	1.57	1.57	1.61	1.79	2.11	1.96	1.43	2.18	1.93
	Reward	1.11	1.61	1.57	1.61	1.75	1.93	2.07	1.75	2.07	1.71
	Mean	1.24	1.61	1.65	1.68	1.83	2.02	2.06	1.68	2.20	1.93
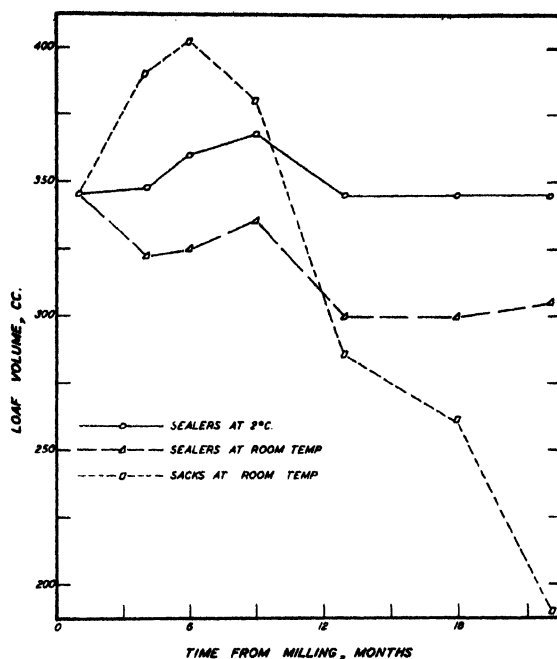
FIG. 1.   *Effect of method and time of storage of flour on the loaf volume of bread.*

of these data are given in Table V.   These results are in striking contrast with the loaf volume results, since time had a very marked effect on acidity of all samples.   The significant interaction between station and variety is largely attributable to the behaviour of Garnet and Marquis, the Edmonton-grown

TABLE V

ANALYSIS OF VARIANCE, ACIDITY

Variance due to	D.f.	Mean squares		
		Sealers at 2° C.	Sealers at room temperature	Sacks at room temperature
Station	1	0.0121	0.0925	0.0365
Variety	3	0.0149	0.0348	0.0291
Time	3	0.3968**	1.3026**	1.0794*ᵃ
Station × Variety	3	0.0813*	0.2095*	0.1553**
Station × Time	3	0.0042	0.0038	0.0754*
Variety × Time	9	0.0073	0.0287	0.0095
Station × Variety × Time	9	0.0156	0.0362	0.0192
Total	31			

* *Significant beyond 5% point.*
** *Significant beyond 1% point.*
ᵃ *Station × Time as error.*
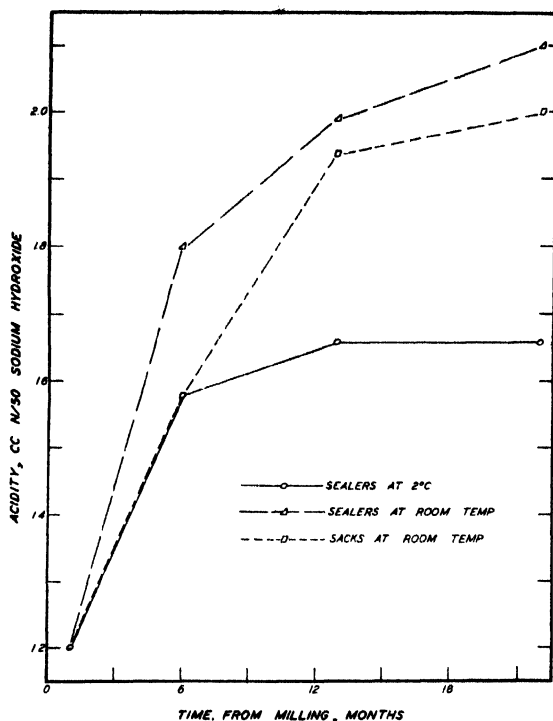
FIG. 2.   *Effect of method and time of storage of flour on acidity.*

sample of the former being very high in acidity, while the acidity of Fallis-grown Marquis was higher than that of any of the other varieties, regardless of conditions of storage.

The differences due to station and variety were not significant under any condition of storage.   The results with flours stored in the refrigerator are of interest because they show that after the first few months of storage, there was very little increase in acidity during the next 15 months.

Discussion

The results presented in this paper substantiate conclusions drawn from earlier work with respect to the effect of various conditions of storage on the keeping properties of flours, but fail to substantiate those concerned with the differential behaviour of flours from different varieties of wheat and different sources.   The results of the earlier work showed that some varieties produced flour that deteriorated much faster than that from other varieties, and that wheat grown on the podsolic loam produced flour of much poorer keeping properties than did wheat grown on the black loam (7).   The failure to confirm these results may be due to the higher than usual protein content of the wheat grown on the podsolic loam, but is more likely the result of some other factor

associated with the very dry growing conditions under which the wheat was produced.

From the practical point of view the results show that flour stored at low temperatures maintains its original quality for long periods, even though the moisture content does not decrease with time. Storage in sacks, the most common method used commercially, is the least effective in preserving quality. Under conditions used in commercial storage, however, the changes which take place in the sacked flour would be much slower than under our conditions. Even under these conditions the quality of the flour was as good as, or better than, it was at the time of milling for at least nine months thereafter. During the interval, this flour made better bread than that stored under either of the other sets of conditions. The original improvement shown by this flour is characteristic of stored flour in general.

The acidity results show that this determination is of very little value in determining either quality, or degree of aging, of flour. These conclusions are in agreement with those reported earlier (7).

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STUDIES IN TREE PHYSIOLOGY

I. GENERAL INTRODUCTION. WATER CONTENTS OF CERTAIN CANADIAN TREES[1]

By R. Darnley Gibbs[2]

Abstract

Previous work by the author on the water contents of Canadian trees is reviewed and followed by a brief discussion of questions yet to be answered.

In *Betula alba v. papyrifera*, in at least the young parts of *B. alba v. pendula laciniata*, in *B. populifolia*, and in several sizes of *Populus tremuloides*, there is a marked seasonal rhythm in water content. The maximum is at leaf opening, the minimum at leaf fall. In poplar but not in birch there is a very high water content in December. During winter a considerable loss of water may occur. A winter loss is shown also by the wood of hemlock and larch and by twigs and leaves of white pine and hemlock. Losses from leaves are surprisingly small.

The behaviour of *B. populifolia* has been studied for more than three years, and differences have been correlated with observations on weather conditions. Experimental work on movement of water in this species during winter is inconclusive. This work continues.

General Introduction

It is estimated that of Canada's 3,457,247 square miles of land, 1,254,083 square miles, or 36.2%, are covered by forest. Not all of this is productive, but the Department of the Interior (2) believes that more than 800,000 square miles have "merchantable" forest; further classification into forest types is still incomplete.

Extensive data dealing with wastage from fire and other causes and with annual increment and cut are available, as might be expected when the value of these natural resources is taken into consideration. Forest pathology, entomology, and genetics (as in the breeding of new fast-growing trees) are coming to the fore and it is, perhaps, true to say that the woods are beginning to receive their due share of scientific attention. Very little is known, however, of the *normal physiology* of Canadian trees, and almost any information on this subject contributes in some measure to an understanding of the problems involved in the efficient utilization of trees.

It is well known that the effects of fungal and insect pests and of difficult climatic and soil conditions upon trees vary in large part with the health of the tree. A full knowledge of the behaviour of the normal tree is therefore necessary to an intelligent study of the "diseased" individual. This is equally true whether the troubles be due to water or salt deficiencies (or excesses), to fungal or insect predators, or to excessive cold.

The writer's introduction to tree physiology was occasioned about 10 years ago by a request from the Canadian Pulp and Paper Association for infor-mation on the seasonal changes in water content of trees. This was needed in connection with efforts to limit the loss of logs by sinkage during flotation. A certain amount of work had been done in Europe by Hartig (12–14), Tonkel (see Büsgen (1)), Geleznow (4), and Craib (3), but the figures were incomplete and it was doubtful if they could be applied safely to Canadian trees. With grants from the Association and from the National Research Council, many water-content determinations were made and the results published in a series of papers (5–9, 11, 16). The trees investigated included paper birch (*Betula alba* var. *papyrifera* (Marsh.) Spach.), aspen poplar (*Populus tremuloides* Michx.), jack pine (*Pinus banksiana* Lamb), white spruce (*Picea canadensis* (Mill.) P.S.P.), larch (*Larix laricina* (Du Roi) Koch), and balsam fir (*Abies balsamea* (L) Mill.). The data, though extensive, were incomplete. They were adequate, however, to reveal the following facts.

a. In the case of paper birch and poplar there is a spring maximum of water content when the trees are practically full of water. This coincides with the swelling and breaking of the buds.

b. Following leaf opening there is a rapid decrease in water content, which continues until August or September. This reduces the amount of water in the tree to little more than half the spring value.

c. After leaf fall the water content rises. The increase is rapid in poplar and proceeds until, at about the end of the year, the tree is again practically full of water. In birch, on the other hand, the increase is much smaller and the tree fills only partially with water during the autumn (7, Figs. 4 and 8).

d. There was some slight indication of a *decrease* in water content during the depth of winter. Few figures were obtained from this period, however, so little definite information as to amount of winter-drying could be given.

e. The *distribution* of water in birch and poplar is consistent with the tension hypothesis of the ascent of sap. This is supported by values for borings at different heights in the tree (8, Table I). Further support is given by the water contents at different heights of individual year-rings in birch, poplar, larch, and spruce (8, Fig. 1).

f. In the softwoods examined (jack pine, white spruce, balsam fir) there is little evidence of seasonal change (7, Tables V–VII).

g. The heartwoods of jack pine and spruce are consistently dry, having practically no *free* water at any time. The heartwood of balsam fir, on the other hand, contains wet patches which may contain more water than the sapwood.

h. Girdling, as a means of reducing water content, is effective only if a complete ring of sapwood be removed. This is not possible in the case of birch and poplar. In the first the whole wood is functional; in the second the sapwood is so wide that all except the very largest of girdled trees would be unstable. Large spruce and balsam may be girdled with success. The wet

patches of the heartwood in girdled balsam, however, do not dry out (at least not in a single season). It was concluded from this that they do not function as water reservoirs. Further investigation of these patches is planned.

i. Superimposed upon the seasonal rhythm is a daily fluctuation, at least in summer (7, pp. 744–6). This fluctuation appears to be relatively small. It is not known for certain whether there is any replacement of broken water columns during these diurnal cycles, but probably only enough water to relieve tension enters the plant during each period of reduced transpiration. Diurnal cycles in diametral changes in trees are well known and have recently been discussed in detail by MacDougal (15). Very little has been done to correlate these with changes in water content, however, though they are no doubt very closely correlated.

j. The behaviour of the tree may vary considerably from year to year (7, Fig. 4).

Enough has been said to show that while the major changes in water content of a few Canadian trees are now fairly well known, there are many points that require further investigation and still others that have not received attention at all. A brief discussion of some of these is in order.

a. Do hardwoods other than paper birch and aspen poplar show a similar seasonal rhythm? May we assume that *all* hardwoods are alike in the general pattern of their behaviour? Are the differences between paper birch and aspen poplar *generic*, *i.e.*, do all species of poplar fill completely with water in the fall and do all species of birch fill only partially during the same period?

b. Do all softwoods behave like those mentioned above or are some like the hardwoods in their behaviour? Larch, with its deciduous habit, should be a particularly interesting tree in this respect.

c. What is the extreme range in behaviour of a single type under varying conditions? This is obviously of the greatest interest for an understanding of "test" years of drought and of extreme cold, for example. It should contribute, too, to our knowledge of the factors determining the climatic and geographic ranges of species. It can help us to distinguish between "abnormal" behaviour, which is due to disease or damage, and the "normal" reaction of the tree to weather and other conditions. When injury *does* occur it may help us to fix the blame and to guard against future damage.

d. How complete is winter rest? Is there any movement of water into and through the tree during the winter? Some investigators seem to believe that there is. The question can be settled without serious difficulty. How much water is lost from the exposed parts of trees during winter? Is this (under Quebec conditions) ever dangerously great? Is there any actual shortage of water in summer, *i.e.*, will irrigated trees behave like those receiving no artificial supply? These questions will be answered if Question *c* be answered.

e. In the case of trees such as aspen poplar and balsam fir, which contain a varying amount of free water in the heartwood, is there *at any time* a utiliza-

tion of that water? One is tempted to conclude from work already carried out that the answer to this question is a flat negative, but the fact is that our information is still too incomplete to warrant such a sweeping assertion. It is relevant to note, in this connection, that in trees such as jack pine and white spruce, which have no free water in the heartwood, the water must have been removed in the transition from sapwood to heartwood.

f. Do young and old trees of the same species behave similarly?

g. So far only the water economy of the tree has been considered. What of other aspects of tree physiology? What foodstuffs are stored and what seasonal changes in these occur? A tremendous amount of work has been carried out elsewhere on a great variety of trees, but many points are still unsettled and there is much to be done before we shall have anything like a complete picture of the behaviour of Canadian trees.

In this and in subsequent papers of this series (one of which is in course of preparation) it is hoped to answer a few of the questions posed above. It is hardly necessary to point out the practical interest of such information, for almost any fact about tree physiology is likely to have a practical value.

Water Contents of Certain Canadian Trees
INTRODUCTION

The work reported in the present paper deals with determinations of water contents. Some of these make more complete the record for trees already studied; others extend investigation to new species. Most observations deal with the wood, but some few apply also to buds and to leaves. As in previous work, oven-drying at 100 to 105° C. has been used, and figures are expressed as a percentage based on dry weight. In all cases weighing of freshly cut pieces was carried out in the field, or samples were placed in weighed, rubber-stoppered vials and weighed on return to the laboratory. It was felt that considerable error might result from oven-drying of coniferous woods and leaves and this has been checked by tetrachloroethylene distillation (10). Except in one or two cases, which are mentioned below, the errors involved were found to be negligible.

1. PAPER BIRCH (*Betula alba* var. *papyrifera* (Marsh.) Spach.)

Figures previously reported (7) for this tree are incomplete, one of the worst gaps in the record occurring in the period of winter "rest". With a view to filling this gap, a number of determinations were made in the spring of 1937 on trees cut on the Price Bros. limits, 40 miles north of Chicoutimi and from the same stand as those used in 1929–31 (7).

The results are given, together with the earlier ones, in Figs. 1 and 5 and in Table VI.

It is clear that there is a distinct, but not large, loss of water from paper birch between December and April and that this is most marked in the upper, smaller parts of the tree. The figures obtained on May 20, 1937, are
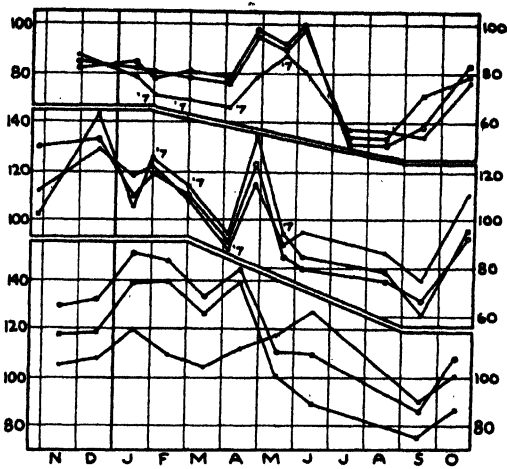
Fɪɢ. 1. *Water contents of paper birch (top) and aspen poplar (middle) from the Price Bros. limits and of aspen poplar (bottom) from Ste. Anne de Bellevue. Results are for discs from "tops" (dots), "middles" (circles), and "butts" (squares). Figures obtained in 1937 are indicated by '7. The curves for aspen poplar from Ste. Anne de Bellevue are averages for three sizes of tree (Table I).*

at first sight most surprising, the average values (six trees) for "top", "middle", and "butt" being 89, 92, and 91%. When these were taken small patches of snow still remained in sheltered places, but the buds were just opening and four of the six trees bled profusely. Evidently these trees were not by any means full of water (they can hold 135% at saturation) although cut at just the time when saturation would be expected. It is almost certain that this indicates a condition like that shown by field birch at Ste. Anne de Bellevue in the same year (see below and Fig. 3), an extremely interesting point and one that should be checked by further work.

2. Wʜɪᴛᴇ Bɪʀᴄʜ Vᴀʀɪᴇᴛʏ (*Betula alba* var. *pendula laciniata?*)

Analyses of buds, ultimate twigs and penultimate twigs from a single isolated specimen of this ornamental birch growing on the McGill campus were made between December 1935 and November 1936 (Fig. 2). The analyses of penultimate twigs are for wood only and so are directly comparable with those for "tops" of paper birch and field birch. In the winter 1935–6, penultimate twigs apparently lost no water or the water was replaced from the older parts of the tree. From mid-March until the end of April a rapid increase in water content occurred, and it is safe to say that at the end of this period the wood was completely filled (the figures are somewhat *above* the average maximum possible water content for wood of paper birch). This coincides with a similar high figure for tops of field birch in the same year. A rapid decrease in water content followed until September, then an increase to an intermediate value. This is very like the behaviour of the other birches.
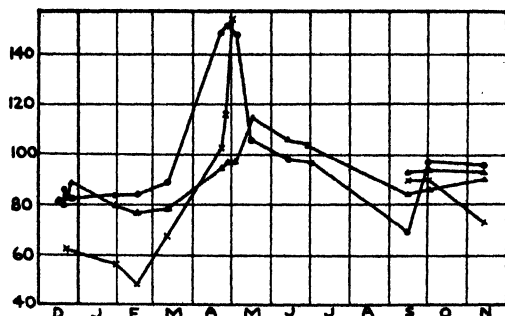
FIG. 2.   *White birch variety: seasonal changes in water content of buds (crosses), wood of penultimate twigs (dots), and entire ultimate twigs (triangles), during 1935–6.*

The figures for ultimate twigs (very slender and pendulous in this tree) are for the whole twig, including cortex, but excluding buds.   Here there is a slight drop in water content during the winter, an increase to a maximum in mid-May, a decrease between then and September and a slight rise in the fall. The ultimate twigs have, of course, become penultimate by that time.

Buds from these shoots lose water in winter (and this loss may in some cases, perhaps, be very serious), then show a rapid gain until at time of opening they have as much water as the wood of penultimate twigs.

3. ASPEN POPLAR (*Populus tremuloides* Michx.)

As in the case of paper birch an effort has been made to supplement the figures obtained for large trees in 1929–31.   The results are summarized in Figs. 1 and 5 and in Table VI.   Here, too, there is a marked drop in water content between December and April.   It is more rapid than in birch and this is in line with the observations on evaporation from the surfaces of the two trees (8, Table II).   The figures obtained on May 20, 1937, are low, but this may really represent in part a rapid fall in water content following leaf opening, for poplar (which is always a little ahead of birch) was just in leaf at this time.

In addition to determinations of water contents in these old trees, a large number of measurements have been made during 1935–8 on trees of three different ages, on the Island of Montreal.   These were all young trees, the oldest have a D.B.H. of 2 to 4 in., the next younger a D.B.H. of 1 to 1½ in., and the youngest (which were almost unbranched) a diameter at the butt of about an inch.   It was hoped that these investigations would give us some idea of the effects of age upon the water economy of the tree, but aspen is perhaps an unfortunate choice for this.   Its water content is found to be extremely variable, and an enormous number of determinations would be necessary before any detailed conclusions could be drawn.   The results are summarized in Tables I and VI and in Figs. 1 and 5.

TABLE I

WATER CONTENTS OF WOOD OF *Populus tremuloides* AT STE. ANNE DE BELLEVUE, 1935–8

Month	Year and part of tree	D.B.H., 2–4 in.		D.B.H., 1–1½ in.		D. at butt *ca.* 1 in.		Averages (No. of trees in parentheses)
January	Year	1936		1936	1937	1936	1937	
	T*	—		—	103	—	99	101 (6)
	T	110		119	135	123	117	119 (15)
	M	164		141	155	153	137	152 (15)
	B	142		145	151	125	129	139 (15)
February	Year	1936		1936				
	T	103		115		- -		109 (6)
	M	159		138		—		149 (6)
	B	143		136		—		140 (6)
March	Year	1936		1936		1936	1936	
	T	86		119		107	104	104 (12)
	M	132		140		135	122	134 (12)
	B	110		139		127	128	126 (12)
April	Year	1936	1936	1936		1936		
	T	97	118	115		113		112 (12)
	M	136	143	151		143		145 (12)
	B	122	129	150		140		139 (12)
May	Year	1936		1936		1936		
	T	112		131		107		117 (9)
	M	86		129		117		111 (9)
	B	75		125		104		101 (9)
June	Year	1936		1936	1936	1936	1936	
	T	129		128	132	126	118	127 (15)
	M	90		120	130	123	106	110 (15)
	B	68		102	98	109	91	89 (15)
September	Year	1936		1936		1936		
	T*	78		78		84		80 (9)
	T	87		90		94		90 (9)
	M	70		96		96		87 (9)
	B	57		79		85		74 (9)
October	Year	1936	1936	1936	1936	1936	1936	
	T*	98	103	88	—	89	97	94 (15)
	T	103	118	103	74	94	114	102 (18)
	M	98	126	111	79	111	124	108 (18)
	B	71	93	90	72	92	106	87 (18)

T* = *Ultimate twigs*, T = *"top"*, M = *"middle"*, and B = *"butt"* as usual.   *Three trees in each group.*

TABLE I—*Concluded*

WATER CONTENTS OF WOOD OF *Populus tremuloides* AT STE. ANNE DE BELLEVUE,
1935-8—*Concluded*

Month	Year and part of tree	D.B.H., 2-4 in.		D.B.H., 1-1½ in.		D. at butt *ca.* 1 in.		Averages (No. of trees in parentheses)
November	Year	1938	1938	1936	1938	1936	1938	
	T*	——	—	105	——	113	—	109 (6)
	T	85	106	131	89	125	92	105 (18)
	M	99	141	148	111	141	113	129 (18)
	B	89	118	150	116	137	96	118 (18)
December	Year	1935	1936	1938	1938	1938	1938	
	T*	—	99	—	——	—	—	99 (3)
	T	115	109	103	116	101	102	108 (18)
	M	141	121	140	138	120	139	133 (18)
	B	127	115	125	124	96	127	119 (18)

T* = *Ultimate twigs*, T = *"top"*, M = *"middle"*, and B = *"butt"* as usual. *Three trees in each group.*

As in the case of the larger trees, there is a rapid and complete refilling of the functional wood in the autumn.   During the period January-March there is some loss of water from the exposed parts of the tree, but this is surprisingly small, particularly in the smallest trees.   It is difficult to understand this.   There is an upswing in water content just before leaf opening, and it is noteworthy that the curve for "top" (2-year-old twigs in these trees) lags behind those for "butt" and "middle", continuing to rise until June. The significance of this will be discussed in a later section.   There is a marked fall in water content during the summer to a low value in September (no figures are available for July and August).

The results, then, are not very different from those for the larger trees, and it is possible to say that after making some allowance for the heartwood in the older trees (it is never very extensive in poplar), the size of the tree has little effect on water content.

4. FIELD BIRCH (*Betula populifolia* Marsh.)

One of the major difficulties in work of this nature is to obtain unlimited supplies of suitable trees in a convenient location.   Through the kindness of Mr. Cleveland Morgan it has been possible to cut about 250 trees of this species from a very uniform stand at Ste. Anne de Bellevue, about 20 miles from Montreal.   These trees average 15 to 20 ft. in height and 2 to 4 in. D.B.H.   The tremendous importance of this accessibility will be realized when it is remembered that not a few of these trees have been cut under appalling conditions in sub-zero weather.   Skis and snowshoes have been used on some occasions.

It is hoped to continue work on field birch for several years in order to get as complete a picture as possible of the effects of environmental conditions upon water economy and to provide a "normal" basis for experimental work. Investigation of this species was started in November 1935 and is still (March, 1939) in progress. The results to date are summarized in Tables II and VI and in Figs. 3 and 5.
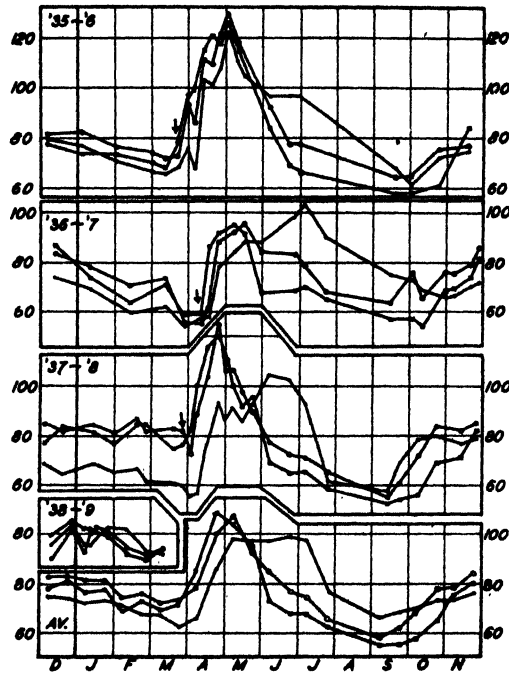
FIG. 3. Water contents of field birch, 1935–9. Results are for "top" (dots), "middle" (circles) and "butt" (squares). The arrows represent times at which soil thawed at a depth of 4 in. (see text and Fig. 4).

Let us consider the general picture as presented by the curve averaging the results for this period.

The water content at the end of the year is about 80% for the whole tree and is not very different in samples from butt, middle, and top. This value is almost exactly the same as that for paper birch at the same time. There is a slow decrease in water content throughout the winter to a low value in mid-March of about 70% for the whole tree. The top is distinctly drier than the butt and middle at this time. A rapid filling of the tree follows, the top lagging somewhat, and on the average, the tree reaches a maximum water content of a little over 100% at the end of April. It is not completely full, however. From the time of leaf opening (the first week of May) a rapid decrease in water occurs, reaching a minimum of about 60% in September. It will be seen that the top again lags, retaining its maximum water content—just below 100%—for two months. At first sight this would seem to be evidence against

TABLE II

WATER CONTENTS OF WOOD OF *Betula populifolia* AT STE. ANNE DE BELLEVUE, 1935–9

Date		Part of tree	Year					Average
			1935	1936	1937	1938	1939	
Jan.	1–15	T	—	77	69	68	75 (6)	72 (15)
		M	—	83	78	84	82 (6)	82 (15)
		B	—	74	74	81	79 (11)	77 (20)
Jan.	16–31	T	—	—	—	65	81	73 (6)
		M	—	—	—	80	80	80 (6)
		B	—	—	—	77	81·	79 (6)
Feb.	1–15	T	—	71	60	—	82	71 (9)
		M	—	76	70	—	72	73 (9)
		B	—	73	63	—	74	70 (9)
Feb.	16–28 or 29	T	—	66	—	63 (9)	72 (4)	67 (16)
		M	—	75	—	84 (9)	70 (4)	76 (16)
		B	—	70	—	84 (9)	72 (4)	75 (16)
Mar.	1–15	T	—	66	62	—	72 (5)	67 (11)
		M	—	71	73	—	74 (5)	73 (11)
		B	—	67	73	—	74 (5)	71 (11)
Mar.	16–31	T	—	72 (6)	55	60 (6)		62 (15)
		M	—	83 (6)	56	82 (6)		74 (15)
		B	—	88 (6)	57	75 (6)		73 (15)
April	1–15	T	—	85 (6)	57	56 (6)		66 (15)
		M	—	99 (6)	56	81 (6)		79 (15)
		B	—	107 (6)	57	91 (6)		85 (15)
April	16–30	T	—	104 (6)	68 (6)	85 (9)		86 (21)
		M	—	115 (6)	75 (6)	111 (9)		100 (21)
		B	—	120 (6)	89 (6)	115 (9)		108 (21)
May	1–15	T	—	117 (6)	84	93 (6)		98 (15)
		M	—	125 (6)	95	102 (6)		107 (15)
		B	—	122 (6)	95	96 (6)		104 (15)
May	16–31	T	—	105	89	93		96 (9)
		M	—	—	93	90		92 (6)
		B	—	—	92	95		94 (6)
June	1–15	T	—	96	87·	104		96 (9)
		M	—	93	85	77		85 (9)
		B·	—	84	67	68		73 (9)
June	16–30	T	—	97	98	102		99 (9)
		M	—	77	82	71		77 (9)
		B	—	69	68	64		67 (9)
July	1–15	T	—	97	103	93		98 (9)
		M	—	78	79	71		76 (9)
		B	—	66	70	65		67 (9)
July	16–31	T	—	—	90[1]	62[2]		76 (6)
		M	—	—	66	63		65 (6)
		B	—	—	65	59		62 (6)

Values are averages for 3 trees except where otherwise indicated in parentheses.

[1] *Obtained by J. H. Whyte and D. Siminovitch.*

[2] *Obtained by D. Siminovitch.*

TABLE II—*Concluded*

WATER CONTENTS OF WOOD OF *Betula populifolia* AT STE. ANNE DE BELLEVUE, 1935-9—
Concluded

Date	Part of tree	Year					Average
		1935	1936	1937	1938	1939	
Sept. 1–15	T	—	—	75	57[2]		66 (6)
	M	—	—	64	53		59 (6)
	B	—	—	57	53		55 (6)
Sept. 16–30	T	—	68	—	69		69 (6)
	M	—	66	—	60		63 (6)
	B	—	58	—	54		56 (6)
Oct. 1–15	T	—	62	71 (6)	75		69 (12)
	M	—	64	71 (6)	72		69 (12)
	B	—	59	56 (6)	55		57 (12)
Oct. 16–31	T	—	73	67	80		73 (9)
	M	—	75	77	83		78 (9)
	B	—	60	68	69		66 (9)
Nov. 1–15	T	—	76	69	76		74 (9)
	M	—	77	76	81		78 (9)
	B	—	84	69	71		75 (9)
Nov. 16–30	T	—	—	71 (6)	80		76 (9)
	M	—	—	83 (6)	83		83 (9)
	B	—	—	78 (6)	82		80 (9)
Dec. 1–15	T	80	74	68	76		75 (12)
	M	80	84	85	80		82 (12)
	B	78	86	77	70		78 (12)
Dec. 16–31	T	—	—	64	83		74 (6)
	M	—	—	81	84		83 (6)
	B	—	—	82	82		82 (6)

Values are averages for 3 trees except where otherwise indicated in parentheses.

[1] *Obtained by J. H. Whyte and D. Siminovitch.*

[2] *Obtained by D. Siminovitch.*

the existence of continuous water columns from butt to top, but an investigation of *distribution* in the tree suggests that this is misleading. More detailed work is desirable, but a few determinations indicate that the outer parts of the butt (which are in direct connection with the wood at the top of the tree) have about the same water content as the top. Thus on July 4, 1938, the average water content at the tops of three trees was 93%, the value for the outer wood at the butt was 85%. The average for the whole butt, however, was only 65%, the distribution from outside to centre being as follows: 85, 55, 62, 64, 68%. It will be remembered that the tops of the small poplars showed a similar lag (Fig. 1). The outer sapwood of hemlock, too, has a high water content at the end of June (Fig. 6). From September until about the end of the year there is a steady increase in water content to about 80%.

The behaviour, then, is very much as in paper birch: a depletion of water content in winter (when uptake is hindered) and in summer (when the tree is in leaf), a refilling (which may be incomplete) in April and in autumn. This is likely, one feels, to be a general picture for hardwoods.

Have other investigators found similar figures? The work of Hartig (12–14), Tonkel (see Büsgen (1)), and Geleznow (4) is not sufficiently complete to make comparison easy. Geleznow's figures, especially, are difficult to reconcile with our own. He gives one value (expressed on a fresh weight basis) for *Betula alba* near Moscow which corresponds with a dry weight figure of about 285%. This might be a possible figure for balsam fir, but it is about double the possible maximum for birch, and one wonders if his other figures are reliable. Tonkel's work is more in line with our own, but he apparently gives no figures for the period March-June and so misses the spring maximum. Hartig's figures are fairly complete. He records a maximum of about 100% for birch at the end of March, a minimum of about 75% in September and a value of around 85% in the middle of winter (see 7, Fig. 4).

Let us consider next the results for individual years. In the winter of 1935–6 there was a slight but steady fall in water content until about mid-March; in 1936–7 similar trees (with about the same water content in November) lost about the same amount of water by mid-March but continued to
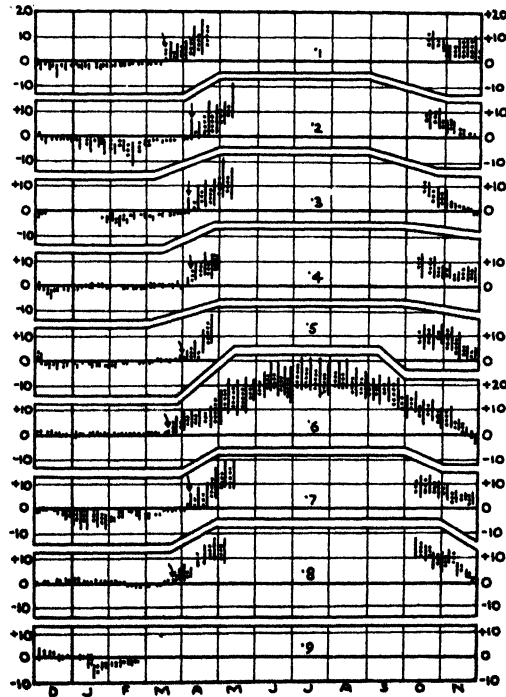
FIG. 4. *Soil temperatures (°C.) at Ste. Anne de Bellevue (Macdonald College) during the period Dec. 1930 to Feb. 1939. Solid lines show weekly range at a depth of 4 in., dotted lines the range at 8 in. Summer figures are omitted for all years but 1936. Arrows indicate probable dates at which soil thawed at 4 in.*

lose water until almost mid-April, while in 1937–8 the trees lost water only in their upper parts, the water content of butts and middles remaining at about 80% (the usual year-end value) until the end of March. During the present winter results seem unusually irregular but the figures suggest a slight loss to date (March 8). Can these differing behaviours be explained?

Fairly complete meteorological data are available (Table IV) from McGill Observatory and St. Hubert Airport. In addition, soil temperatures at 4 and 8 in. have been recorded at Ste. Anne de Bellevue (Fig. 4). Soil temperatures

TABLE III

PHENOLOGICAL AND OTHER DATA

Observations	Year				Remarks
	1936	1937	1938	1939	
Maple sap flow at Ste. Anne de Bellevue	—	Mar. 12	Mar. 24	V. slight, Mar. 12 – 14 and 23–24.	Same trees. Not necessarily the earliest date.
First week with mean air temp. over 40° F.	Mar. 16– 22	April 8– 24	Mar. 18–24		McGill Observatory.
Thawing of soil at 4 in. Thawing of soil at 8 in.	Mar. 20 Mar. 23	April 6 April 9	Mar. 27 Mar. 29		Macdonald College, Ste. Anne de Bellevue. Fig. 4.
General soil temperature during winter	High	Low	High: lower at end	High first half; low second half.	Fig. 4.
Loss of water by field birch in winter	Steady but slight	Large	Very slight except in twigs	Slight; irregular to Mar. 8	Fig. 3.
Increase in water content of field birch	ca. Mar. 15	ca. April 15	ca. April 4		Fig. 3.
Alder catkins expanded Alder catkins dehiscing	Mar. 31 —	April 10 April 17	April 9 April 16		
Earliest maple in flower	—	April 15	April 7–8		Same tree.
Field birch bleeding	Mar. 31* April 25	April 17 April 27	April 16 April 23		*Stumps of cut trees.
Field birch catkins expanded Field birch catkins dehiscing	May 2 Before May 10	— May 8	April 30 After May 5		
Bark of field birch slipping:— At top At butt	 Before April 25? May 10	 April 27 May 8–16	 April 23 May 5		
Buds of field birch showing ca. ⅛ in. of green	—	May 8	April 30		
Max. water content at butt and middle of field birch	ca. 130% ca. May 2	ca. 95% ca. May 8	ca. 123% ca. April 23		Fig. 3.

in 1935–6 and in 1937–8 were generally high, remaining at or a little above 0° C. for much of the time. Definite increases and daily fluctuations in soil temperatures, representing thawing at 4 and 8 in., occurred between March 20 and 23 in 1936 and March 27 and 29 in 1938. These dates correspond very closely with the first increases in water content recorded in those years (Table III). It is obvious, therefore, that something other than soil temperature is responsible for the different behaviours of the trees during these two winters. In 1937, soil temperatures were very low from early December until late March, and the soil finally thawed at 4 and 8 in. between April 6 and 9. This was reflected in the behaviour of field birch, for in this year all parts of the trees lost water until almost mid-April. During the present winter, soil

TABLE IV

ABSTRACT OF METEOROLOGICAL DATA

Observations	Month	1935–1936	1936–1937	1937–1938	1938–1939
Mean temp. (°F.) for month, and range of daily means (in parentheses) (McGill Observatory)	Dec.	16 (−1 to 35)	29 (5 to 42)	19 (−3 to 32)	24 (2 to 41)
	Jan.	13 (−10 to 34)	24 (1 to 41)	15 (−5 to 38)	15 (−9 to 42)
	Feb.	12 (−2 to 36)	23 (7 to 39)	17 (−1 to 35)	16 (−2 to 29)
	Mar.	33 (8 to 48)	24 (8 to 35)	27 (−3 to 49)	
Relative humidity (McGill Observatory)	Dec.	79	72	60	80
	Jan.	82	77	75	79
	Feb.	77	77	79	81
	Mar.	72	70	82	
Rain (in.) Snow (in.) Rain and melted snow (in.) (McGill Observatory)	Dec.	0.3 14 1.8	2.4 19 4.4	0 37 4.3	2.1 15 3.9
	Jan.	0.5 34 4.3	3.8 12 5.7	0.9 15 2.4	1.3 21 3.4
	Feb.	0.2 31 3.6	1.1 15 2.6	1.6 18 3.7	0.5 39 5.1
	Mar.	5.2 13 7.3	0 36 3.5	1.4 17 3.7	
Percentage possible sunshine (McGill Observatory)	Dec.	24	25	28	21
	Jan.	26	27	31	30
	Feb.	46	33	34	28
	Mar.	30	31	37	
Mean vel. of wind (miles per hr.) (St. Hubert Airport, Que.)	Dec.	10	13	12	13
	Jan.	12	15	12	14
	Feb.	14	12	11	16
	Mar.	12	13	11	

temperatures have been low after a very mild December.   This record is incomplete at the time of writing.

Let us consider for a moment the question of movement of water into and through the tree during the winter months, for this might explain the data obtained.   It is certain that the trees investigated are frozen for a part of the time, but it is also a matter of observation that they thaw during mild weather, even when the air temperature is somewhat below freezing point. It is quite possible that a slow movement of water *through* the tree can take place under these conditions, but can water *enter* the tree?

This question cannot yet be answered with certainty, but a few experimental results (summarized in Table V) may be considered here.

On January 16, 1937, several small branches were cut from trees of field birch at Ste. Anne de Bellevue and analyzed.   Entire ultimate twigs of these (*i.e.*, those formed the previous summer) had 73% of water, while the penul-

TABLE V

WATER CONTENTS OF AMPUTATED PARTS OF FIELD BIRCH

Expt. nos. and dates	Part cut and treatment given	Entire penultimate twig	Entire ultimate twig	Remarks
S.A. 37.4, Jan. 16, and S.A. 37.13, Mar. 29.	Freshly cut branches, Jan. 16	75	73	There was no recorded increase in water content of the trees as a whole until after April 11. Cut ends of branches painted and wrapped in rubber.
	Similar branches, cut Jan. 16, left tied to trees until Mar. 29, then analyzed.	38	37	
	Freshly cut branches, Mar. 29	57	67	
S.A. 37.7, Feb. 13, and S.A. 37.13, Mar. 29.	Freshly cut branches, Feb. 13	63	72	
	Similar branches, cut Feb. 13, left tied to trees until Mar. 29, then analyzed.	47	47	
	Freshly cut branches, Mar. 29	54	67	

Expt. nos. and dates	Part cut and treatment given	Butt	Butt[1]	Middle	Top	Remarks
S.A. 38.7, Feb. 26 and S.A. 38.12, April 1.	Freshly cut trees (6) Feb. 26	84	—	83	61	[1]Cut midway between butt and middle.
	Trees (3) cut Feb. 26, left guyed in position on stump until April 1, then analyzed.	—	59	68	55	[2]High values probably partly due to spring increase. See text.
	Freshly cut trees (3), April 1	82[2]		73[2]	55	
S.A. 39.2, Jan. 11, S.A. 39.3, Jan. 14, and S.A. 39.9 Mar. 8.	Freshly cut trees, Jan. 11 (3) and 14 (2).	81[3]	—	81	75	[3]Includes butt values of trees cut and guyed.
	Trees cut Jan. 11 (3) and 14 (2), left guyed in position on stump until Mar. 8, then analyzed.	—	76	76	71	Cut ends painted and wrapped in rubber.
	Freshly cut trees (5), Mar. 8	74	75	74	72	

timate twigs had 75%. Exactly similar branches were cut at the same time, their ends were painted and tightly wrapped in rubber, and they were then tied to the trees in the positions they had occupied. Ten weeks later (on March 29) they were analyzed, as were freshly cut "control" branches. They were found to contain 37% and 38% of water in ultimate and penultimate twigs, while the "controls" had 67% and 57% respectively. A second set was treated similarly from Feb. 13 until March 29. In this case, too, the detached twigs had lower water contents than those still attached to the tree (47% and 47% against 67% and 54%). It would seem from this that water had moved into the attached twigs, but this may have happened just prior to March 29, though it should be emphasized that no increase in water content of the trees as a whole was registered until after April 11.

A similar experiment was carried out in 1938, but in this case whole trees were cut, the ends sealed and the trees guyed in their original positions. They were cut on February 26 and analyzed on April 1. This experiment was inconclusive as the control trees cut on April 1 were already taking up water. However, it is clear that the *tops* of the detached trees were no drier than those of the controls, which probably had received none of the water taken up from the soil in the few days prior to cutting.

In the present year a further experiment with cut trees gave very uniform results, the water contents of three trees cut on Jan. 11, and two on Jan. 14, and left guyed in position until March 8 being almost exactly the same as those of attached trees freshly cut on March 8. Here there is absolutely no evidence of movement *into* the control trees; but on the other hand there has been remarkably little loss either from detached or from attached trees during the seven weeks of almost continuously cold weather. It is obvious that a much more extended series of experiments is needed, and these are planned for the next winter.

Are some of the differences in winter behaviour of field birch to be attributed to differing rates of evaporation during the winters studied? Apparently no agency measures evaporation during the winter, so no comparative figures are available. A consideration of the meteorological data given in Table IV permits of no very definite conclusions. Temperatures in 1937–8 were between those of 1935–6 and 1936–7; humidity was at first lower, then higher than in those years; wind was a little lower; sunshine generally higher. This would not point to greatly reduced evaporation in 1937–8.

The most likely explanation of the high figures for water in butts and middles of field birch during the winter of 1937–8 would seem to be uptake of water from the soil, but as we have seen above this did not appear to occur in 1935–6, although soil-temperature conditions were similar.

It may be a matter of coincidence, but during these three winters the dates of thawing of the soil, of a weekly mean air temperature of 40° F., and of first increases in water content have shown a close correspondence (Table III).

The spring rise in water content during 1936 lasted from mid-March until the beginning of May—about six weeks—and resulted in an increase from

about 70% to nearly 130%, at which figure the trees were nearly saturated. In 1937 the increase occurred during about a month (mid-April to mid-May) —except in "tops"—and resulted in an increase from about 57% to about 94%. It seems that the trees had time to fill only partially in this year before the leaves opened. It will be remembered that paper birch from above Chicoutimi had a water content of about the same value at about the same time (Fig. 1). In 1938, when the increase started at about the end of March and continued until barely a month later, the water content rose almost to the 1936 figure of 130%, but this was from a "low" of about 80%.

The outer parts of the trees (and hence the figures for "tops") seem always to lag somewhat behind the inner parts (and hence behind the averages for "butts" and "middles"). This is particularly noticeable in the record for 1937, when the maximum for the tops was 103% in early July; but the behaviour in 1938 was similar and even in 1936, when the lag was least obvious, a fairly high water content persisted in the tops until the end of June.

The phenological and other data given in Table III are admittedly incomplete, but they indicate that although the spring of 1937 started late there was little difference between the years 1936, 1937, and 1938 by the end of April. An examination of the soil temperatures given in Fig. 4 further shows that the spring of 1937 was about as late as any between 1931 and 1939, while those of 1936 and 1938 were about as early as any. In short we have struck, in all probability, the approximate limits for this season in the years under consideration.

In the three years for which figures are available the late summer minimum has been about the same. Figures for this season are not sufficiently numerous, however, to make accurate comparisons possible. It is doubtful if the trees suffered from any real shortage of water in the summers studied, because the month by month precipitation from May to September in 1936 was 4.1, 2.9, 4.7, 4.2, and 2.2 in. In the same period of 1937 it was 4.5, 4.0, 5.3, 3.5, and 3.1 in., while for 1938 it was 3.7, 3.4, 3.5, 5.8, and 6.5 in. It would be interesting, nevertheless, to investigate the effects of irrigation upon water content, and it is hoped to do this in the near future.

In each of the four years for which December figures have been obtained a water content of about 80% is indicated.

Weight and Density of Freshly Cut Hardwoods

It was mentioned in a previous section of the present paper that early work on water contents was carried out in connection with the sinkage problem. It is interesting to see how the more extensive figures now available for seasonal changes in water content apply to flotation and transportation.

A cubic foot of water weighs 62.5 lb. If a cubic foot of freshly cut wood weighs 62.5 lb. it will just float—i.e., its density is 1.0. Poplar with this density would contain about 138% of water: paper birch about 100%. Any decrease in water content from these figures would result in a corre-

TABLE VI

WATER CONTENTS, DENSITIES AND WEIGHTS OF FRESHLY CUT HARDWOODS

Time of cutting	Aspen poplar (old trees; D.B.H. about 8 in.; 40 mi. N. of Chicoutimi)			Aspen poplar (young trees D.B.H. 1–4 in.; Island of Montreal)			Paper birch (old trees; D.B.H. about 8 in.; 40 mi. N. of Chicoutimi)			Field birch (young trees, D.B.H. 2–4 in.; Island of Montreal)		
	Water content (% based on dry weight)	Density (water = 1.0)	Weight, lb. per cu. ft.	Water content (% based on dry weight)	Density (water = 1.0)	Weight, lb. per cu. ft.	Water content (% based on dry weight)	Density (water = 1.0)	Weight, lb. per cu. ft.	Water content (% based on dry weight)	Density (water = 1.0)	Weight, lb. per cu. ft.
January	111	0.89	56	145	1.03	63	82	0.91	57	79	Cannot be calculated accurately from our records but will compare closely with paper birch.	
February	111	0.89	56	145	1.03	63	80	0.90	56	74		
March	–	–	–	126	0.95	60	–	–	–	72		
April (early)	92	0.82	51	140	1.01	62	78	0.88	55	81		
April (late)	124	0.95	59				91	0.95	59	103		
May	90	0.80	50	108	0.87	55	91	0.95	59	99		
June	–	–	–	100	0.83	53	92	0.96	60	80		
July	87	0.78	49	–	–	–	54	0.77	48	70		
August	81	0.76	47	–	–	–	54	0.77	48	–		
September	68	0.70	43	80	0.75	47	62	0.81	51	59		
October	98	0.83	52	98	0.83	52	80	0.90	56	68		
November	–	–	–	122	0.93	59	85	0.93	58	79		
December	135	0.99	62	125	0.94	60	–	–	–	81		

Maxima and minima in all cases in bold face type.

sponding decrease in weight and a greater margin of flotation.   Increased flotation is important in the "driving" of logs, while decreased weight is obviously of great importance for haulage.   It will be seen from Table VI and Fig. 5 how these figures change with the season in the case of birch and
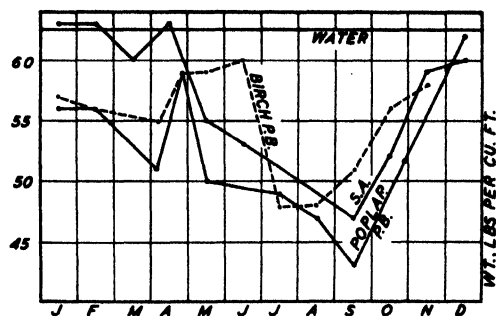
FIG. 5.   *Seasonal changes in weight of freshly cut wood of paper birch and aspen poplar from Price Bros. limits above Chicoutimi and of aspen poplar from Ste. Anne de Bellevue.*

poplar*.   From them we learn that a cubic foot of young poplar cut in January may weigh 63 lb. (*i.e.*, it will sink at once if placed in water), while wood from the same tree cut in September will have a density of about 0.75 and will weigh more than 25% less.   Birch shows similar changes.   This is obviously of considerable practical interest and our knowledge of such facts should be extended to all commercial species.

5. HEMLOCK (*Tsuga canadensis* Carr.)

Between November, 1936 and June, 1938, 46 young hemlock trees, averaging a little over 5 in. D.B.H., were cut at Ste. Anne de Bellevue.   These grew in a mixed woodland very near the stand of field birch described in the previous section.   The results from these trees are summarized in Fig. 6 and in Table VII.

Hemlock, like poplar, varies greatly from tree to tree, the sapwood being relatively narrow and the heartwood (as in balsam fir) having wet and dry patches.   This variability makes it necessary to cut a large number of trees in order to get really accurate figures, and the 46 trees used here are not enough for detailed conclusions.   It is clear, however, that a marked seasonal variation in water content occurs in the sapwood, and that this parallels closely the behaviour of the outer wood of birch and poplar.   There is a high water content at the end of the year, a distinct drop throughout the winter and a rise to a maximum in June.   This is followed by a decrease during the summer to a low value in September or October and an increase from then until December.   Since these changes are restricted to the sapwood, and since this is relatively narrow, the water contents of these trees *as a whole* show little change; older trees would show even less.

* *Some of these data have already been published (9).*

TABLE VII

WATER CONTENTS OF HEMLOCK AT STE. ANNE DE BELLEVUE, 1936-8

Date (No. of trees in parentheses)	Expm't nos.	Discs			Distribution											
					Butt				Middle				Top			
					Sapwood		Heartwood		Sapwood		Heartwood		Sapwood		Heartwood	
		Butt	Middle	Top	Outer	Inner	Outer	Inner	Outer	Inner	Outer	Inner	Outer	Inner	Outer	Inner
January (4)	37.2 37.5 38.1	100	112	122	128	100	84	95	143	114	88	101	149	119	94	77
February (5)	37.6 37.8 38.4 38.5	88	101	114	102	69	80	104	125	88	86	97	143	102	90	99
March (6)	37.9 37.11 37.12 38.8 38.9	93	107	113	122	89	74	87	136	113	86	99	130	104	108	94
April (4)	37.14 37.16 37.18	78	90	113	84	69	79	82	104	71	89	107	136	96	100	101
May[1] (4)	37.20 37.22 37.23	87	87	103	87	63	91	111	107	73	72	104	121	77	106	104
June[2] (6)	37.24 37.25 37.26 37.27 38.21	94	104	118	123	81	80	98	135	101	84	84	150	104	97	94
September (4)	37.30 37.31	76	101	118	84	56	65	115	107	81	114	108	136	107	108	107
October (4)	37.33 37.34	86	91	104	97	74	85	102	113	83	80	87	122	92	84	99
November (5)	36.35 37.37 37.38 37.39	94	113	124	112	81	89	95	134	108	98	116	147	112	108	101
December (4)	36.37 36.39 37.42	104	119	139	134	100	76	95	152	111	101	90	167	122	122	98

[1] Includes two trees cut June 1.
[2] Includes one tree cut July 6.

TABLE VIII

WINTER WATER LOSSES OF LARCHES, PINES, HEMLOCK, AND DOUGLAS FIR

Date	Larix laricina — Three trees, D.B.H. 5–6 in., Price Bros. limits above Chicoutimi, 1936–7 — Sapwood (Av. of tops, middles, and butts)	Larix europaea — One tree, ca. 8 ft. high, at McGill, 1938–9 — Wood of 2-year old twigs[1]	Larix europaea — Entire 1-year old twigs[2]	Pinus strobus — Old trees at Ste. Anne, 1936–7 — Entire 2-year old twigs	Pinus strobus — Entire 1-year old twigs	Pinus strobus — Leaves[3]	Pinus ponderosa — Trees near Pullman, Washington, U.S.A. (Clements*), 1936–7 — Leaves of current year	Pinus ponderosa — Leaves 1-year old	Pinus ponderosa — Leaves 2-year old and older	Tsuga canadensis — Old trees at Ste. Anne, 1936–7 — Entire 2-year old twigs	Tsuga canadensis — Entire 1-year old twigs	Tsuga canadensis — Leaves[2]	Pseudotsuga mucronata — Trees near Pullman, Washington, U.S.A. (Clements*), 1936–7 — Leaves of current year	Pseudotsuga mucronata — Leaves 1-year old	Pseudotsuga mucronata — Leaves 2-year old and older
Nov. 6	—	126	112	104	133	141	—	—	—	106	93	138	—	—	—
Nov. 7	—	—	—	—	—	—	114	109	100	—	—	—	138	118	102
Nov. 26	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Dec. 5	—	—	—	118	131	139	—	—	—	96	75	137	—	—	—
Dec. 23	—	122	107	—	—	—	—	—	—	—	—	—	—	—	—
Dec. 27	—	126	108	—	—	—	126	114	111	—	—	—	137	121	106
Dec. 31	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Jan. 16	—	—	—	108	129	142	—	—	—	82	69	134	—	—	—
Jan. 25	—	114	105	—	—	—	—	—	—	—	—	—	—	—	—
Jan. 27	—	113	105	—	—	—	—	—	—	—	—	—	—	—	—
Jan. 31	135	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Feb. 5	—	—	—	—	—	—	125	110	104	—	—	—	139	121	103
Feb. 14	—	117	103	—	—	—	—	—	—	—	—	—	—	—	—
Feb. 28	123	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Mar. 5	—	—	—	96	106	130	123	111	104	83	63	132	130	116	101
Mar. 12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Mar. 17	—	106	97	—	—	—	—	—	—	—	—	—	—	—	—
Mar. 28	—	103	100	—	—	—	—	—	—	—	—	—	—	—	—
April 4	107	102	107**	117	144	142	98	89	87	91	72	139	102	87	78
April 8	—	—	—	116	133	142	—	—	—	86	75	129	—	—	—
April 10	—	106	105	—	—	—	—	—	—	—	—	—	—	—	—
April 20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
May 20	121	—	—	—	—	—	—	—	—	—	—	—	—	—	—

[1,6,8] Results high by 2, 6, and 8% as indicated by distillation figures. Figures given were obtained by oven drying.

* Clements, H. F. Research Studies State Coll. Wash. 6 : 3-45. 1938. Clements' figures have been recalculated on a dry weight basis.

** After rain and wet snow.

Minimum in bold face type.

It should be remembered that results for jack pine, spruce, and balsam previously reported (7) do *not* indicate a similar seasonal cycle, but the figures for these were not sufficiently extensive to rule out *all* change and some re-examination of those trees is planned.
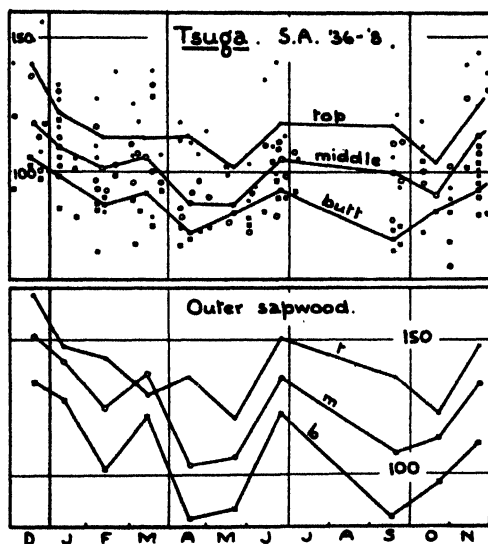
FIG. 6. *Water contents of hemlock at Ste. Anne de Bellevue 1936–8. Above—results from individual trees and averages for discs from tops, middles, and butts (dots, circles, and squares respectively). Below—water contents of outer sapwood.*

In addition to the observations on hemlock that are noted above some measurements have been made of the water contents of twigs and leaves of this species during the winter of 1936–7. These results, together with similar ones for white pine, some winter figures for *Larix europaea* and *L. laricina* and some figures obtained by Clements for *Pinus ponderosa* and *Pseudotsuga mucronata* in the State of Washington, are summarized in Table VIII.

In all these trees there is a definite loss of water during the winter. The loss is surprisingly small—less than 10%—in the coniferous leaves examined in Canada, but may be quite large—about 32%—in twigs of hemlock. Clements' figures indicate very little loss before early March, but a sharp drop between then and April 8. A similar drop may occur in the Canadian trees (no analyses were made between March 12 and April 10, but by the latter date the trees were obviously taking up water). Further investigation obviously is desirable.

Acknowledgments

This paper is an interim report so little needs to be said by way of conclusion. It remains to thank those who have made the work possible. Financial aid—by way of a research grant—has been received from the National Research Council, and some of the earliest figures were obtained

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